NATIONAL CERTIFICATION SYSTEM FOR TISSUE CULTURE RAISED PLANTS (NCS-TCP)

Plant Tissue Culture: Techno-Commercial Feasibility

Department of Biotechnology
Ministry of Science & Technology
Govt. of India

Biotech Consortium India Limited
New Delhi
PART A: PRIMER ON PLANT TISSUE CULTURE
1. Introduction

A whole plant can be regenerated from a small tissue or plant cells in a suitable culture medium under controlled environment. The plantlets so produced are called tissue-culture raised plants. These plantlets are a true copy of the mother plant and show characteristics identical to the mother plant. For example, if the mother plant is a high yielding plant the plantlets will also be high yielding. Many plant species are presently being propagated through tissue culture successfully.

This capacity of a single cell to grow into a complete plant is termed as Totipotency, which was first put forward by a German Botanist Haberlandt in 1902. Tissue culture is the propagation of plants wherein a part/tissue of the plant is placed in nutrient media that favors the production of shoots, roots following which they are hardened and transferred to soil. Quality planting material of economically important species can be produced in a large scale/desired quantity through tissue culture.

Plant tissue culture can be initiated from almost any part of a plant however, for micropropagation or direct shoot regeneration, meristemetic tissue such as shoot tip is ideal. The physiological state of the plant does have an influence on its response to tissue culture. The mother plant must be healthy and free from obvious signs of disease or pest. The shoot tip explants being juvenile contain a higher proportion of actively dividing cells. It is important to use quality mother plant stock to initiate cultures.

The cultural conditions required to initiate and sustain plant cells in culture, or to regenerate intact plants from cultured cells, are different for each plant species. Each variety or clone of a species often have a particular set of cultural requirements.
2. Process of Plant Tissue Culture

- Excised explants taken from mother plant are treated to eliminate microbes
- Preparation of sterile media
- Explants are inoculated under sterile bench/laminar air flow
- Inoculated explants are kept in growth rooms under controlled conditions of light and temperature
- Frequent monitoring for contamination
- Cutting and multiplying the shoots (sub-culturing) under sterile conditions
- Incubation in growth room
- Rooting and incubation
- Hardening and planting in the field
3. Stages of Tissue Culture Process

3.1 Preparation of nutrient medium

A semi-solid medium is prepared in double distilled water containing macro elements, micro elements, amino acids, vitamins, iron source, carbon source like sucrose and phyto-hormones. The medium is heated for dissolving the agar and 25 to 50 ml is dispensed into each wide mouth bottles. The vessels containing culture media are then sealed and sterilized by autoclaving.
3.2 Establishment of aseptic culture

The starting material for the process is normally an actively growing shoot tip of axillary or terminal bud or shoot tip of a plant. The process of tissue culture starts from the selection of mother plants having the desired characteristics. Ex-plant preferably the meristematic tissue of the selected mother plant is isolated. The excised tissue/explant is washed with water and then rinsed with a disinfectant such as savlon or detol solution followed by a sterile-water wash. The tissue is then dipped in 10% bleach solution for ten minutes for disinfecting the plant tissue material, killing most of the fungal and bacterial organisms. Sterilization process of explants depends on the plant species and types of explants.

3.3 Inoculation

Inoculation is carried out under aseptic conditions. In this process explants or micro shoots are transferred on to the sterilized nutrient medium.

![Fig II: Inoculation of excised micro shoots](image)

3.4 Development of plants in growth room

After the inoculation of the plant tissue, the bottles are sealed and transferred into growth room to trigger developmental process under diffused light (fluorescent light of 1000-2000 lux) at 25 ± 2°C and 50 to 60% relative humidity. Light and temperature requirements vary from species to species and sometimes during the various stages of developments.
The cultures are observed daily for growth and any signs of infection/contamination. Cultures, that do not show good growth or infected, are discarded. The healthy cultures grow into small shoot buds. These are sub-cultured on the fresh medium after 4 weeks. The number of subcultures required is specific to the plant species, which are standardized. The shoots generally develop after 4 weeks. After enough number of shoots is developed in each container (10 to 15), to a minimum height of 2 cm they are transferred to another medium for initiating the process of rooting. The constituent of rooting medium for each plant species are specific. Roots are generally formed within 2 to 4 weeks. Plants at this stage are delicate and require careful handling.

![In vitro rooting of micro shoots](image)

**Fig III: In vitro rooting of micro shoots**

### 3.5 Hardening of micro plants

Due to very high humidity inside the culture vessel and artificial conditions of development, the plantlets are tender and are therefore are not ready for coping up with the filed conditions. The plants removed from the sterile medium are washed and are maintained under intermittent mist or are covered with clean transparent plastic. After 10 to 15 days under high humidity, the plants are transferred to green house and maintained for another 4 to 6 weeks. They are then ready to be transferred to net house or the field. Normally, the tissue culture plants are sold either as ex-agar plants or hardened plants from the green house.
3.5.1: Ex-agar plants

Depending on the parameters such as location/the site of planting, soil quality and the climatic conditions defined by the customer, the ex-agar plant for sale could be *in vitro* rooted plants or only the shoots. When the tissue culture plants are sold at this stage, the plants are washed in sterilized water to remove the agar medium.

The washed plants are sorted into 2 to 3 grades and packed in corrugated plastic boxes lined with sterilized tissue paper as per specifications of the Plant Quarantine Authority, Government of India for exports. The number of plants per box depends on the customer’s requirement. Depending on the final destination and the preference of the customer, the plants are treated with specific fungicides and antibiotics to avoid infection.

The ex-agar plants are preferred for export or for destinations where hardening facility are available. The plants after being removed from nutrient media should preferably be transplanted within 72 hours.

*Fig IV: Ex agar plants ready for packaging and dispatch*
3.5.2: Hardened plants

The plants are transferred to net pots/pro tray for acclimatization after they fully develop shoots and roots in the bottles. The rooted plantlets are transferred to pots filled with suitable substrate and are watered. This operation is carried out on an open bench. These pots are then transferred to the green house for 4 to 6 weeks. During this process, they are given fertilizers and treated like plantlets obtained by any other means of propagation. After the plants are acclimatized fully, they are transferred to poly-bags. At this stage the plants are completely hardened and are ready to be planted in the field for cultivation. Hardening units can be set up in sites away from the micropropagation unit.

Fig. V: Hardening of plants in green house

4. Advantages of Micro-propagation Technology

Micro-propagation has several advantages over conventional methods of propagation such as:
1. **Rapid multiplication**: 
   Micro-propagation offers rapid multiplication of desired plant species.

2. **Requirement of only limited number of explants**: 
   Small pieces of plant (explants)/tissue can be used to produce a large number of plants in a relatively small space.

3. **Uniform or true to type plants**: 
   Micro-propagation provides a high degree of phenotypic/physical uniformity. Since the production cycle takes place under controlled conditions, proper planning and scheduling based on the market demand is possible. The resulting product has very high degree of uniformity compared with traditionally propagated plants.

4. **Germplasm storage**: 
   Plants can be stored *in vitro* in a small space and less labour is required for maintenance of stock plants.

5. **Disease free planting material**: 
   Plantlets produced by tissue culture are usually disease free. With proper diagnosis and treatments, elimination of fungus, bacteria and virus prior to large scale propagation is possible. With the help of serological and molecular technique it is possible to index virus of mother plant/explant which is to be used for mass multiplication.
6. **Growth manipulation:**
Nutrient levels, light, temperature and other factors can be more effectively controlled to manipulate the growth, multiplication and regeneration.

7. **Round the year production:**
Micro-propagation is independent of season. As micro-propagation could be carried out throughout the year; production cycle can be scheduled to meet peak demands.

8. For species that have long generation time, low levels of seed production, or seeds that do not readily germinate, rapid propagation is possible through tissue culture.

9. The time required is much shortened, no need to wait for the whole life cycle of seed development.

5. **Commercially propagated plants through micro-propagation in India**
The plants in each category which are commercially propagated are as follows

<table>
<thead>
<tr>
<th>Plant category</th>
<th>Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruits</td>
<td>Banana, Pineapple, Strawberry,</td>
</tr>
<tr>
<td>Cash crops</td>
<td>Sugarcane, Potato</td>
</tr>
<tr>
<td>Spices</td>
<td>Turmeric, Ginger, Vanilla, Large cardamom,</td>
</tr>
<tr>
<td></td>
<td>Small Cardamom</td>
</tr>
</tbody>
</table>
**Medicinal plants**
Aloevera, Geranium, Stevia, Patchouli, Neem

**Ornamentals**
Gerbera, Carnation, Anthurium, Lily, Syngonium, Cymbidium

**Woody plants**
Teak, Bamboo, Eucalyptus, Populus

**Bio fuel**
Jatropha, Pongamia

6. **Mitigating Risks of commercial plant tissue culture**

The utilization of plant tissue culture for commercial production is limited by two major risks viz., spread of diseases especially those caused by viruses, and variations. The movement of plants also involves accidental risk of introducing plant disease. Pathogens that are often symptom less, such as viruses, pose a risk. The risk of distribution of inferior micropropagated plants has posed a major threat to the ever-increasing agribusiness industry. In order to prevent these risks, effective testing (indexing) procedures are required prior to bulking up culture for commercial propagation. Standard procedure should be adopted such as:

- Carefully selection of mother plants
- Ensuring establishment of virus free culture through indexing of 100 % explants
- Proper package and practices to be adopted such as limited number of cycles of multiplication, grading of cultures as well as plants, insect, pest monitoring in hardening area etc.

7. **Need for Certification of tissue culture raised plants**

Micropropagation is effectively used for producing quality planting material free from disease. Yet there is threat of inadvertent propagation of virus
infected plants which will not only result in loss or poor performance of the crop but also spread of virus. Further failure to used standard crop specific guidelines can lead to variations in the plants produced. The most deleterious variants in tissue culture raised plants are those that affect yield through somaclonal variations and carry viruses and other pathogens which are difficult to diagnose. This is an area of great concern and requires a well structured system to support the tissue culture industry to ensure virus free quality planting material for commercial production.

With the objective of production and distribution of quality tissue culture planting materials Department of Biotechnology (DBT), Government of India has established National Certification System for Tissue Culture Raised Plants (NCS TCP). For details about NCS-TCP, please refer the manual on “National Certification System for Tissue Culture Raised Plants (NCS-TCP): An Overview or log in to www.dbtnctscp.nic.in. DBT is the Certification agency for the purpose for certification of Tissue culture raised plants/propagules up to laboratory level and to regulate its genetic fidelity as authorized vide the Gazette of India Notification dated 10th March 2006 of Ministry of Agriculture under section 8 of the Seeds Act, 1966.
PART B: TECHNO-COMMERCIAL FEASIBILITY
1. MARKET SCENARIO

Demand for tissue cultured plantlets is growing rapidly. India, with its low cost skilled labour as well as scientific manpower (both of which are essential for tissue culture) has a natural advantage. Additional favourable factors are the wide range of plant biodiversity in the country and favorable tropical climate (which enables greenhouses with low energy consumption).

The potential for the domestic market is enormous and by conservative estimates it is around Rs. 200 crores with an annual growth rate of 20%. There are more than 70 established commercial tissue culture units. Their production capacity ranges between 0.5 million to 10 million plants per annum with an aggregate production capacity of about 200 million plantlets per year. The protocols have either been developed in-house or transferred through the various research institutions and universities engaged in development of the protocols through support of the Department of Biotechnology (DBT).

Currently, the focus of the companies is mainly banana, floriculture, sugarcane and potato.

With increasing awareness about the advantages of tissue culture raised plants in improving yield and quality, their domestic consumption is also increasing optimistically. The major consumers of tissue culture raised plants are the State Agriculture Department, Agri Export Zones (AEZs), State agencies such as Spice Board, sugar industry and private farmers. The paper industry, medicinal plant industry and State Forest Departments are using tissue culture raised plants in a limited scale. Also a number of progressive farmers and nurseries in the states are the major consumers of Tissue culture plants particularly for flowers, banana, sugarcane and medicinal plants.

2. Establishment of Commercial Plant Tissue Culture Unit

Commercial plant tissue culture unit consists of the following components.
Storage room for chemicals:

It is advisable to have a separate area for storage of chemicals, apparatus and equipments. Chemicals required in small amounts should not be purchased in large quantities as they may lose their activity, pick up moisture or get contaminated. Such problems can be overcome by purchasing small lots on a regular basis.

Washing and Media Preparation Room:

The glassware washing area should be located near the sterilization room. This area should have at least one large sink but two sinks are preferable with running tap water. Adequate workspace is required on each sides of the sink; this space is used for glassware soaking and drainage. Plastic netting can be placed on surfaces near the sink to reduce glassware breakage and enhance water drainage. The outlet pipe from the sink should be of PVC to resist damage from acids and alkalis. Both hot and cold water should be available and the water still and de-ionisation unit should be located nearby. The washing room should be swapped periodically. Mobile drying racks can be used and lined with cheesecloth to prevent water dripping and loss of small objects. Ovens or hot air-cabinets should be located close to the glassware washing and storage area. Dust-proof cabinets and storage containers should be installed to allow for easy access to glassware. When culture vessels are removed from the growth area, they are often autoclaved to kill contaminants and to soften semi-solid media. It should be possible to move the vessels easily to the washing area. The glassware storage area should be close to the wash area to expedite storage and access for media preparation.

The media preparation room should have smooth walls and floors, which enable easy cleaning to maintain a high degree of cleanliness. Minimum number of doors and windows should be provided in this room
but within the local fire safety regulations. Media preparation area should be equipped with both tap and purified water. An appropriate system for water purification must be selected and fitted after careful consideration of the cost and quality. A number of electrical appliances are required for media preparation; hence, it is essential to have safety devices like fire extinguisher, fire blanket and a first aid kit in the media preparation room. A variety of glassware, plastic ware and stainless steel apparatus is required for measuring, mixing, and media storage. These should be stored in the cabinets built under the worktables and taken out for use as and when required. The water source and glassware storage area should be in or near the media preparation area. The workbench tops should be made with plastic laminate surfaces that can tolerate frequent cleaning. Media storage room should have capacity to storage the media for at least 7 days. Sterility Class 1,00,000 is desirable for media storage room.

**Inoculation Room**

The most important work area is the Inoculation room where the core activity takes place. The transfer area needs to be as clean as possible with minimal air disturbance. Walls and floors of the Inoculation room must be smooth to ensure frequent cleaning. The doors and windows should be minimal to prevent contamination, but within local safety code. There is no special lighting requirement in the transfer room. The illumination of the laminar airflow chamber is sufficient for work. Sterilization of the instruments can be done with glass-bead sterilizers or flaming after dipping in alcohol, usually ethanol. The culture containers should be stacked on mobile carts (trolleys) to facilitate easy movement from the medium storage room to the transfer room, and finally to the culture room. Fire extinguishers and first aid kits should be provided in the transfer room as a safety measure. Special laboratory
shoes and coats should be worn in this area. Ultraviolet (UV) lights are sometimes installed in transfer areas to disinfect the room; these lights should be used only when people and plant material are not in the room. Sterility Class 1,00,000 is desirable for inoculation room which can be achieved through installation of pressurized air module or air handling unit.

**Growth Room**

Culture room is an equally important area where plant cultures are maintained under controlled environmental conditions to achieve optimal growth. It is advisable to have more than one growth room to provide varied culture conditions since different plant species may have different requirements of light and temperature during *in vitro* culture. Also, in the event of the failure of cooling or lighting in one room, the plant cultures can be moved to another room to prevent loss of cultures. In the growth room, the number of doors should be minimal to prevent contamination. The culture containers can be placed on either fixed or mobile shelves. Mobile shelves have the advantage of providing access to cultures from both sides of the shelves. The height of the shelves should not exceed 2m.

The primary source of illumination in the growth room is normally from the lights mounted on the shelves. Overhead light sources can be minimized, as they would be in use only while working during the dark cycle. Plant cultures may not receive uniform light from the conventional downward illumination. Lights directly fitted to the racks create uneven heat distribution. Sideways illumination is an alternative, which requires less number of lights, and provides more uniform lighting. But care has to be taken not to break the lights while moving the cultures across the shelves. Sterility Class 1,00,000 is desirable for growth room.
3. PROJECT DETAILS

A: PROFILE OF A SELF CONTAINED UNIT:

The project profile of a micropropagation unit with an annual production capacity of 3 million plantlets is discussed below. A product mix of 5 different plants has been assumed:

1. Banana \( \textit{Musa acuminata} \)
2. Sugarcane \( \textit{Saccharum officinarum} \)
3. Ginger \( \textit{Zingiber officinale} \)
4. Medicinal plants \( \textit{Chlorophytum borovillianum} \) (Safed musli), \( \textit{Aloe barbadensis} \)
5. Ornamental plants Carnation-\( \textit{Dianthus caryophyllus} \), Orchids-\( \textit{Vanilla} \)

Location

The tissue culture laboratory should be preferably located in a moderated climate condition having uninterrupted supply of water and power. The tissue culture operations have to be carried out under controlled conditions of temperature. Extreme climatic condition adds to the cost of maintenance.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Head</th>
<th>Cost ( Rs. In lakhs)</th>
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<tbody>
<tr>
<td>1.</td>
<td>Land</td>
<td>5.00</td>
</tr>
<tr>
<td>2.</td>
<td>Land development</td>
<td>5.60</td>
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<tr>
<td>3.</td>
<td>Building</td>
<td>35.20</td>
</tr>
<tr>
<td>4.</td>
<td>Utilities</td>
<td>16.00</td>
</tr>
<tr>
<td>5.</td>
<td>Equipment</td>
<td>69.40</td>
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</tbody>
</table>
6. Green and shade house 30.00
7. Miscellaneous fixed asset 2.75

**Total** 163.95

**Land:** Approximate 5 acres land should be adequate for setting up a TC unit with the above capacity. Cost of land is assumed at Rs. 5.00 Lakhs

**Building and civil works**
The building of about 8800 sq.ft includes class 1000 clean rooms and areas with comfort AC for laboratory, growth rooms and office space.
The following facilities would be required in the building.

a) Storage room for chemicals
b) Washing and Media preparation room
c) Sterilization room
d) Inoculation room
e) Culture room

The total cost is estimated at Rs. 35.20 lakhs @ Rs. 400/sft.

**Green house**
A green house of 7500 sq.ft. and a shade house of 80,000 sq.ft. have been assumed at a cost of Rs. 22.00 lakhs and 8.00 lakhs (total Rs. 30 lakhs) respectively. The greenhouse should be provided with heating equipment, fans and cooling systems.

**Equipment**
Major equipment and instruments required for the plant are as follows.

- Autoclave
- Laminar air flow cabinet
- Equipment for sterilization
- Electronic weighing balance
- Water distillation apparatus
Air handling units
Refrigerator
Air conditioners
Stereomicroscope
Digital pH meter
Shelves / racks
Green house material

WORKING CAPITAL REQUIREMENT

(I) Raw material
The basic inputs for the production of micropropagated plantlets include meristems of elite and disease free plants, ready to use culture medium, sucrose and agar.

(II) Manpower
The unit with the proposed capacity may need 40-50 people at various positions including managerial, supervisory, skilled and unskilled

(III) Recurring expenses (per month)

<table>
<thead>
<tr>
<th>(Rs. lakhs)</th>
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<tbody>
<tr>
<td>Raw Material</td>
</tr>
<tr>
<td>Manpower</td>
</tr>
<tr>
<td>Utilities (power, water)</td>
</tr>
<tr>
<td>Contingencies (marketing, office expense, repair etc)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

Recurring expenses (per annum) Rs. 69.12 lakhs

CAPITAL INVESTMENT

<table>
<thead>
<tr>
<th>(Rs. lakhs)</th>
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<tbody>
<tr>
<td>Fixed assets</td>
</tr>
<tr>
<td>Technology knowhow</td>
</tr>
<tr>
<td>Working Capital (3 months)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>
MEANS OF FINANCE

Particulars                                           (Rs. in Lakhs)
1. Debt                                               117.73
2. Equity                                             78.50

Total                                                196.23
Debt: Equity                                         3:2
Interest                                             16%

FINANCIAL ANALYSIS

(I) Cost of Production

Recurring cost (per annum)                           Rs. 69.12
Depreciation (@10%)                                   Rs. 17.87
Interest (@16% Pa)                                   Rs. 18.83

Total                                                Rs. 105.82

(II) Turnover

Average selling price                                Rs. 7 per plant
Total no. of plants                                  30 lakhs

Total turnover                                        Rs. 210 lakhs

(III) Profitability

Net profit                                           Rs. 104.18 lakhs
% Profit on sales                                     49%
IRR                                                   26%
Return on investment                                 53%
(B) ECONOMICS OF STARTING PLANT TISSUE CULTURE BUSINESS WITH THE MINIMAL INVESTMENT

Micro propagation business can be started by entrepreneurs interested in venturing into this area, with smaller investment by setting up a hardening unit to start with. Such entrepreneurs can procure primary hardened tissue culture plantlets from established micro propagation units and undertake secondary hardening in the facility and sell it to the farmers. Once the market is established, a full-fledged micro propagation unit could be set up. The following profile provides an overview of profitability for a hardening facility for handling 3 lakh plantlets per annum.

HARDENING FACILITY

Project Details

Capacity
3 lakh plantlets / annum

Land
1 acre

Project Cost

A. FIXED ASSETS

<table>
<thead>
<tr>
<th>S. No</th>
<th>Heads</th>
<th>Rs in lakhs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Land and site development</td>
<td>1.00</td>
</tr>
<tr>
<td>2.</td>
<td>Green House</td>
<td>8.00</td>
</tr>
<tr>
<td>3.</td>
<td>Electrical fittings</td>
<td>0.60</td>
</tr>
<tr>
<td>4.</td>
<td>Furniture and fixtures</td>
<td>0.60</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>10.20</strong></td>
</tr>
</tbody>
</table>
B. Recurring expenses (per month) (Rs. lakhs)
- Raw Material (Rs 4.00/explant) 1.00
- Manpower 0.15
- Utilities {power (500 units), water} 0.15
- Contingencies 0.05
- Total 1.35

Annual Recurring Expenses (per annum) Rs. 16.20 lakhs

C. CAPITAL INVESTMENT (Rs. lakhs)
- Fixed Assets 10.10
- Working capital (3 months) 3.63
- Total 13.73

D. MEANS OF FINANCE

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<table>
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<tr>
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<tbody>
<tr>
<td>1.</td>
<td>Debt</td>
<td>10.25</td>
</tr>
<tr>
<td>2.</td>
<td>Equity</td>
<td>3.63</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>13.73</td>
</tr>
</tbody>
</table>

Debt : Equity - 3:2
Rate of interest on loans - 16%

E. FINANCIAL ANALYSIS

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Particulars</th>
<th>(Rs. Lakhs)</th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td>Debt</td>
<td>10.25</td>
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<td>3.63</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>13.73</td>
</tr>
</tbody>
</table>
(I) Cost of Production (per annum) (Rs. lakhs)

- Recurring cost 14.52
- Depreciation (@10%) 0.80
- Interest (@16%) 1.31

Total 16.63

Debt : Equity 3:2

(II) Turnover

- Total plantlets 3 lakhs
- Selling price Rs. 8 per plant

Total turnover Rs. 24 lakhs

(III) Profitability

- Net profit - Rs. 7.37 lakhs
- % Profit on sales - 30%
- IRR - 19%
- Return on investment - 56%

4. Government Schemes and Incentives

Various Central and State Government departments have framed financial schemes and announced incentives for assistance of tissue culture industry which are summarized below:

a. Ministry of Agriculture

The Department of Agriculture and Cooperation under the Ministry of Agriculture, Government of India has the following programmes and schemes for promotion of horticulture.
(i) There is a provision for assistance of upto Rs. 21 lakhs and Rs. 10 lakh for setting up tissue culture units in public and private sector respectively subject to a maximum of 20% of the project cost.

(ii) Under the Integrated Development of Fruits scheme assistance is given for purchase of planting material under the area expansion programme for the following crops:

a) Rs. 7,000/hectare for plants of Guava, Amla, Date Palm, Plum Peach, Bes, Fig and citrus.

b) Rs.10,000/hectare for plants of mango, almond, pomegranate, apple, nuts, apricot, olive, papaya, litchi and sapota.

c) Rs. 30,000/hectare for plants for Bananas and pineapples.

d) Rs. 70,000/hectare for plants of grapes and strawberry.

In addition, 50% subsidy is given to the farmers for purchase of tissue culture banana by the Andhra Pradesh State Agriculture Department under the Macro Management Scheme.

b. Agricultural and Processed food products Export Development Authority (APEDA)

APEDA under the Ministry of Commerce and Industry has taken the following initiatives for promoting tissue culture in the country.

(i) A state-of-the-art airfreight trans-shipment centre has been set up for temperature sensitive perishables at Delhi, Mumbai and Bangalore airports.

(ii) Airfreight subsidy is given for Tissue Culture Plants along with other live plants / bulb in category of perishable horticulture produce for export. The rate of subsidy to West Asia and CIS countries is at the rate
of Rs.10 per kg or 25% of the airfreight rate approved by IATA or 1/3rd of the FOB value whichever is the least.

(iii) The rate of subsidy for export to Europe other than CIS countries, North America and Far East at the rate of Rs.25 per kg or 25% of the airfreight rate approved by IATA or 1/3rd of the FOB value whichever is the least.

(iv) 50% subsidy is given for the development of infrastructure like refrigerated van, packaging, export promotion, market development, consultancy services and feasibility studies, organization building and human resource development.

(v) Financial assistance is also given for strengthening quality control facilities and implementation of ISO 9000.

c. National Horticulture Board (NHB)

The mandate of NHB is to promote integrated development of Horticulture and to help in coordinating, stimulating and sustaining the production and processing of fruits and vegetables. It also helps in establishing a sound infrastructure in the field of production, processing and marketing with a focus on post harvest management. For setting up of a new tissue culture lab there is a provision for back-ended capital subsidy not exceeding 20% of the project cost with a maximum limit of Rs. 25 lakh per project. NHB also has a scheme for providing subsidy for cultivation under controlled climate condition in poly houses, green houses, net houses, etc. The units planning expansion in the domestic market by having a network of nurseries or additional hardening facilities can avail this scheme. The provision also exits for high quality commercial horticulture crops, Indigenous crops/produce, herbs, aromatic & medicinal plants, seed & nursery, bio-pesticide and establishment of Horticulture Health Clinics/
Laboratory. In all these cases, the subsidy is routed through the involvement of a financial institution on the completion of the project.

d. Department of Biotechnology (DBT)

DBT supports R & D projects across the country at the various laboratories in the universities and the research institutions for development and standardization of tissue culture protocols for various species through tissue culture. DBT has supported 150 projects so far for development of micropropagation related protocols for about 50 plant species. DBT established National Certification System for Tissue Culture Raised Plants vide the Gazette of India Notification dated 10\textsuperscript{th} March 2006 of Ministry of Agriculture under \textbf{section 8 of the Seeds Act, 1966} for the purpose of certification of Tissue culture raised plants up to laboratory level and to regulate its genetic fidelity.

e. Small Farmers Agri business Consortium (SFAC)

SFAC under the Ministry of Agriculture give soft loans up to 50 lakhs for setting up of small tissue culture labs by cooperative societies formed by small scale farmers.

f. State Level Incentives

The states of Karnataka, Gujarat, Maharashtra and Andhra Pradesh are giving financial assistance for setting up tissue culture labs under the new agro industrial policy. Karnataka gives capital subsidy of 20\% on investments in setting up tissue culture unit whereas the subsidy is 6\% in Gujarat. Maharashtra gives a subsidy on power consumption. In addition, state government provides subsidy to the farmers for purchase of tissue culture plants under various schemes.
g. **Financial Assistance by Banks**

Apart from the fiscal incentives given by the central and state governments, the financial institutions have also been financing tissue culture projects as a priority sector. Some nationalized banks like Canara Bank has opened a special cell for financing high tech agriculture projects. National Bank for Agriculture and Rural Development (NABARD) under its refinancing scheme has supported some 30 projects.
PART C: FREQUENTLY ASKED QUESTIONS (FAQs)
PART C: FREQUENTLY ASKED QUESTIONS (FAQS)

What is Plant Tissue Culture?

It is the process of producing plants from tissues of the desired plant in an artificial nutrient medium under controlled environment. The plants so produced would be exactly similar to the mother plant in all aspects.

Why one should go for tissue culture plants?

Tissue culture plants are qualitatively better as they are produced under aseptic condition and controlled environment. One should go for tissue culture plants for the following reasons.

- Tissue culture raised plants are vigorous and fast growing than conventional plants
- High degree of uniformity than the conventionally produced plants
- The tissue culture plantlets are free from diseases/pathogens
- It is possible to multiply plants that are difficult to propagate by cuttings or other traditional methods
- Tissue culture plants yield better as they are produced under optimum environment from selected mother plants.

How plant tissue culture/ micro propagation is done?

A small piece of plant tissue (explant) is taken from the donor plant and cultured on a nutrient medium in sterile containers. By altering the composition of the medium and the environmental conditions (temperature, light regime, etc.) the development of this piece of tissue can be directed along different patterns and finally the whole plant can be regenerated. The offspring comes from a single plant and are thus identical to each other and to the mother plant.
Whether tissue culture plants are more prone to pest and diseases than plants propagated conventionally?

The tissue culture raised plants are free from disease and pest, if the standard procedures are adopted for production. Tissue culture plants grow faster and better, yet they are as vulnerable as any other plants unless appropriate precautions are taken. However, tissue culture plants are free from soil borne diseases.

Do tissue culture plants yield better than the conventional plants?

As tissue culture plants are uniform, vigorous and disease free, if proper cultivation practices are properly followed, the total yield is expected to be better compared to conventional plants.

How can we ensure that the plants produced from tissue culture are free from viruses and other pathogens?

To ensure quality and disease free distribution of tissue culture plants National Certification System for Tissue Culture Raised Plants has been established by Department of Biotechnology, Ministry of Science and Technology, Government of India. Under this system tissue culture plants are tested for viruses as well as quality at different Test Laboratories accredited by the system. The plants that are free from viruses and good quality get a Certificate of Quality. This system also recognizes the Tissue Culture Production Unit based on guidelines for infrastructure, expertise, and technical management and package & practices for production. Hence, procuring certified tissue culture plants from recognized tissue culture production unit ensures quality as well as free from virus and diseases.
What is the role of Biotech Consortium India Limited (BCIL) under NCS-TCP?

BCIL is the company promoted by the Department of Biotechnology, Government of India to facilitate accelerated commercialization of biotechnology. The major areas of activity include technology transfer, consultancy, information dissemination, manpower training, Intellectual Property Protection and project management. BCIL is closely associated with tissue culture industry and various stages of evolution of NCS-TCP. BCIL is the Project Management Unit and Accreditation Unit of NCS-TCP.

**Accreditation Unit (AU):** As the Accreditation Unit, BCIL has been assisting DBT in Accreditation of Test Laboratories and Recognition of Tissue Culture Production facilities

**Project Management Unit (PMU):** As the Project Management Unit (PMU), BCIL is coordinating the several components and various activities under NCS-TCP

**Who will certify the tissue culture raised Plants?**

DBT has established Accredited Test Laboratories (ATLs) for virus indexing and quality testing of tissue culture raised plants and issue a Certificate of Quality on behalf of Certification Agency i.e. DBT.

ATLs will perform both test i.e. virus indexing and quality testing

At present, there are 9 Test Laboratories where these test are carried out.

- National Research Centre for Banana, Trichi, Tamil Nadu
- Central Potato Research Institute, Shimla, Himachal Pradesh
- Indian Institute of Spice Research, Calicut, Kerela
- Indian Institute of Horticulture Research, Bangalore, Karnataka
- Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh
- University of Agricultural Sciences, Bangalore, Karnataka
- Sugarcane Breeding Institute, Coimbatore, Tamil Nadu
- Agarkhar Research Institute, Pune and Indian Agriculture Research Institute, Regional Station, Pune (jointly)
- Central Research Institute for Jute & Allied Fibres, Barrackpore

**Who can get their planting material certified from Accredited Test Laboratories**

Only recognized tissue culture companies under NCS-TCP are eligible to get their planting material certified from Accredited Test Laboratories (ATLs).

**Which are the Referral Centres under NCS-TCP**

DBT has identified two national laboratories namely Indian Agriculture Research Institute (IARI), New Delhi (for virus indexing) and the Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad (for genetic fidelity testing) as Referral laboratories to update the ATLs with respect to recent testing protocols. These referral centres also provide trainings to ATLs from time to time on the updated testing protocols.

**What is the duration of validity of Certificates of Recognition and Accreditation?**

The validity of Certificate of Accreditation and Recognition is 2 years which is subject to renewal.

**How the NCS-TCP is dynamic and continuous evolving?**

NCS-TCP is very dynamic system which is continuous evolving in the light of experience gained during the course of its implementation.
Apex Committee, Project Monitoring and Evaluation Committee (PMEC) and Accreditation Panel are the committees, which comprise of a pool of experts in the area for guiding and monitoring the progress of NCS-TCP. Guidelines are revised from time to time. Crop specific standards for tissue culture plants have been developed for many economical plant species such as potato, banana, sugarcane, vanilla, apple, bamboo, citrus and black peeper. Standards for remaining plant species are under development. Referral Centres of NCS-TCP provide updated protocol and time to time training to Accredited Test Laboratories.

For any further query related to plant tissue culture, please feel free to contact:

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