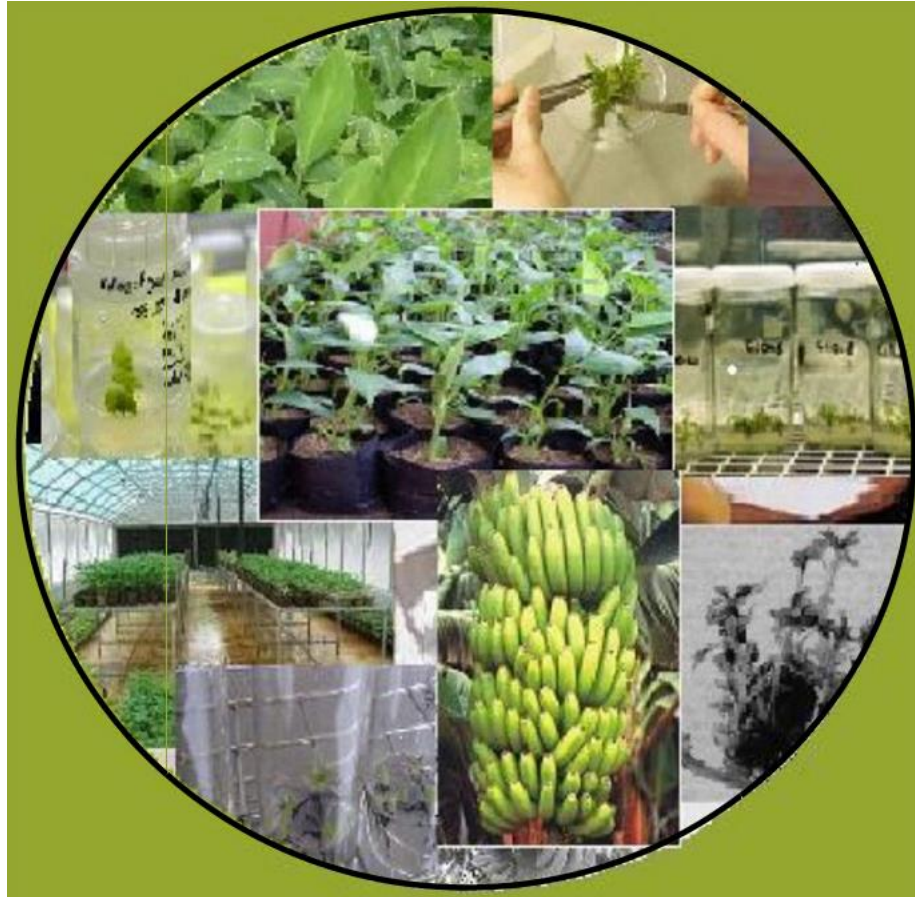


PROJECT REPORT ON
Plant Tissue Culture



NODAL TRAINING INSTITUTE

Regional Institute of co-operative Management

SUBMITTED BY

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Agri Clinics & Agri Business Center Scheme

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HIGHLIGHTS OF THE PROJECT REPORT

A. ABOUT THE PROMOTER

- a. Name : Maya Sawant
- b. Address : At/P- Harali Kh.,Tal –Gadhinglaj, Dist- Kolhapur 416502
- c. Contact Number : 9767504701
- d. Date of Birth : 13-01-1981
- e. Educational Qualification : M.Sc. (Botany)
- f. Experience : Fresher

A. FIRM PROFILE

- g. Name of the Firm : Tissue culture Laboratory
- h. Address : At/P- Harali Kh.,Tal – Gadhinglaj,Dist-Kolhapur 416502
- i. Constitution : Proprietorship

A. PROJECT PROFILE (FINANCIAL)

PARAMETERS	VALUES
1. Type of Project	Tissue Culture Laboratory
2. Unit size in sq.m. Medium Scale	600 Sq ft
3. Product	Plants
4. Cost of the project	15,82,960
5. Bank loan	15,82,960
6. Margin money Land	Land
7. Financial Indicators BCR at 20% DF 1.81:1	1.41
NPW 15%	11,42,519
IRR (%) 77.70%	14.36%
DSCR 18.85	3.22

8. Interest rate (% per annum)	12%
9. Repayment period	5 Years

2. Project Description

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, meristematic 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

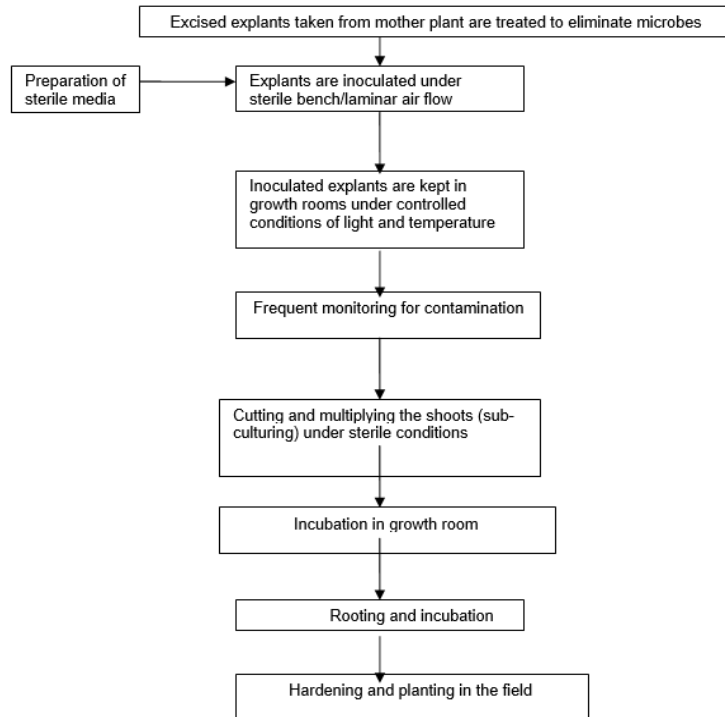


Figure I: Production process of Tissue Culture Plants (TCPs)

3. Stages of Tissue Culture Process

a. Preparation of nutrient medium

A semi- sealed 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 and sterilized by autoclaving.

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

b. 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

c. 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 $25 \pm 2^{\circ}\text{C}$ 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.



Fig III: In vitro rooting of micro shoots

d. Hardening of microplants

Due to very high humidity inside the culture vessel and artificial conditions of development, the plantlets are tender and are therefore not ready for coping up with the field 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 greenhouse 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 greenhouse.

i. Ex-agarplants

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 72hours.



Fig IV: Ex agar plants ready for packaging and dispatch

ii. : 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:

i. Rapid multiplication:

Micro-propagation offers rapid multiplication of desired plant species.

ii. 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.

iii. 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.

iv. Germplasm storage:

Plants can be stored *in vitro* in a small space and less labour is required for maintenance of stockplants.

v. 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.

vi. Growth manipulation:

Nutrient levels, light, temperature and other factors can be more effectively controlled to manipulate the growth, multiplication and regeneration.

vii. 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.

viii. 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.

ix. 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

Plant category

Plants

Fruits	Banana, Pineapple, Strawberry,
Cashcrops	Sugarcane, Potato
Spices	Turmeric, Ginger, Vanilla, Large cardamom, Small Cardamom
Medicinal plants	Aloevera, Geranium, Stevia, Patchouli, Neem
Ornamentals	Gerbera, Carnation, Anthurium, Lily, Syngonium, Cymbidium
Woody plants	Teak, Bamboo, Eucalyptus, Populus
Biofuel	Jatropha, Pongamia

1. 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.

1. Banana *Musa acuminata*

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.

MICROPROPAGATION OF BANANA SWOT ANALYSIS

1. STRENGTH:

Agro climatic condition is suitable for tissue culture banana Cultivation.

Bunches are uniform, big finger size and average 140-150 fingers per bunch.

Financing institution are also interested to finance of commercial production of banana tissue culture.

2.WEAKNESS :

It requires more investment in initial stage.

It is relatively sophisticated technology than suckers and need to be well equipped with knowledge and skills.

3.OPPORTUNITY :

Heavy market demand with good price.

The enterpreneur could get subsidy and credit facilities .

4.THREATS :

There will be heavy damage to the plant if there is cyclone and hailstorm.

Complete watch and ward is necessary for discriminates for storing bunches.

Heavy winter during bunch initiation period reduces the bunch size and occurrence of throat choke disease.

A. PROJECT COST

1. CAPITAL COST

PARTICULARS	UNIT	QUAN TITY
I. Technical Parameters		
1. Number of batches	No.	8
2. Rack for storage	No.	10
3. Chemical's	Type s	10
4. Glass wears	Type s	6
5. Labour requirement per batchs	Hrs	24
II. Economic Parameters		
1. Cost of construction of tissue cuture room(15 x 20 ft)	Rs/s q.ft.	250
2. Cost rack	Rs/ra ck	2,500
3. Cost on glass bottle	Rs/B ott	200
4. Cost Chemicals	Rsba tch	20
5. Labour wage rate	Rs./h rs	30
6. Yield per Batches	Nos	5,000
7. Sale Price	Rs/k g	2

PARTICULARS	UNIT	UNI T RATE Rs	QUANTIT Y	AMOUN T Rs.
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I. Capital Cost

1. Land and Site Development

Land				1,00,00 0
Site development	Ls.			1,00,00 0
2. Building				
Media storage room	Sq.ft	250	80	20,000
Culture transfer room	Sq.Ft.	250	40	10,000
Growth room	Sq.Ft.	250	100	25,000
Laboratory	Sq.ft	250	50	12,500
Office room	Sq.ft	250	30	7,500
3. Machinery & Equipment's				
	Ls.			8,00,00 0
4. Furniture				
	Ls.			2,00,00 0
5. Green house				
	Sq.ft	250	500	1,25,00 0
6. Metal Rack				
	Nos	0	250	10
				25,000
7. Insurance				
	Kg	800	100	80,000
5. Contengencies				
	%	5		25,000
			TOTAL-A	15,30,000

II. Working Capital (One Crop Cycle requirement)

1. Chemicals	Kg.	800	100	5,000
2. Bags	Rs./No	1	0	1600
3. Raw material	Lumpsum			5,000
4. Electricity + Water	Lumpsum			10,000
6. Miscellaneous Exp	Lumpsum			16000
8. Labour charges	Hrs	30	24	960
9. Marketing expenses	Lumpsum			5,0 00
10. Transportation	Lumpsum			1,0 00

			TOTAL (B)	52,960
TOTAL COST OF PROJECT			(A+B)	15,82,960

C. MEANS OF FINANCE

PARTICULARS	UNIT	UNIT RATE	AMOUNT Rs.
1. Term loan	%	85	13,45,516
2. Own contribution	%	15	2,37,444
TOTAL			15,82,960
3. Subsidy entitlement @44% from NABARD under AC & ABC Scheme			696502.4

PARTICULARS	UNIT	UNIT RATE	QUANTITY	IYEAR	IIYEAR	IIIYEAR	IVYEAR	VYEAR
I. INCOME								
Capacity utilized	%			100	100	100	100	100
Yield per Batch	Nos	4	6,40,000	25,60,000	25,60,000	25,60,000	25,60,000	25,60,000
Interest on subsidy @ 6%					41,790	41,790	41,790	-
Subsidy				-	-	-	-	6,96,502
			TOTAL	25,60,000	26,01,790	26,01,790	26,01,790	32,56,502
1. Chemical		800		40,000	40,000	40,000	40,000	40,000
2. Bags		1		6,40,000	6,40,000	6,40,000	6,40,000	6,40,000
3. Raw material	Ls			5,000	5,000	5,000	5,000	5,000
4. Electricity + water	Ls			10,000	10,000	10,000	10,000	10,000
5. Marketing	Ls			5,000	5,000	5,000	5,000	5,000
8. Labour charges /cycle	960		8	7,680	7,680	7,680	7,680	7,680
10. Transportation /cycle	1,000			1,000	1,000	1,000	1,000	1,000
			TOTAL	7,08,680	7,08,680	7,08,680	7,08,680	7,08,680
III. NET INCOME				18,51,320	18,93,110	18,93,110	18,93,110	25,47,822

F. FINANCIAL ANALYSIS

PARTICULARS	I YEAR	II YEAR	III YEAR	IV YEAR	V YEAR	TOTAL
Capital costs	15,30,000					
Recurring costs	4,23,680	4,23,680	4,23,680	4,23,680	4,23,680	
TOTAL COST	19,53,680	4,23,680	4,23,680	4,23,680	4,23,680	
Benefit	18,51,320	18,93,110	18,93,110	18,93,110	25,47,822	
Depreciated value of buildings @10%					7,500	
Depreciated value of equipments & furniture @15%					15,000	
TOTAL BENEFIT	18,51,320	18,93,110	18,93,110	18,93,110	25,47,822	
NET BENEFIT	-1,02,360	14,69,430	14,69,430	14,69,430	21,24,142	
Discounting Factor @15%	0.87	0.76	0.66	0.57	0.5	
NPV cost at 15% DF	16,99,702	3,21,997	2,79,629	2,41,498	2,11,840	27,54,665
NPV benefits at 15% DF	-89,053	11,16,767	9,69,824	8,37,575	10,62,071	38,97,184
NPW at 15% DF	11,42,519					
BCR at 15% DF	1.41					
IRR%	14.36					

Rate of interest 12%
Opening balance of term loan **15,82,960**

Sr No	Loan O/S	Net income	Principal	Interest	Total Repayment	Net surplus	DSCR
1	15,82,960	-1,02,360	3,16,592	1,89,955	5,06,547	14,44,327	-0.20
2	12,66,368	14,69,430	3,16,592	1,51,964	4,68,556	18,54,517	3.14
3	9,49,776	14,69,430	3,16,592	1,13,973	4,30,565	21,46,507	3.41
4	6,33,184	14,69,430	3,16,592	75,982	3,92,574	21,68,496	3.74
5	3,16,592	21,24,142	3,16,592	37,991	3,54,583	28,15,807	5.99
						Average DSCR	3.22

