PROJECT PROFILE

ON

TISSUE CULTURE

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TISSUE CULTURE

1. Introduction

The success of Horticulture development hinges on selection of desired types of plants and their multiplications. Selection of desired types is based on evaluation of the quantitative and qualitative performance of plants and also in some cases their aesthetic appeal. Over the years, the horticulturists have developed various techniques for selection of desired types of plants and their multiplication. Recently interesting developments have taken place in the field of plant multiplication which involves culture of cells or tissues in laboratory.

Traditionally, horticultural plants are multiplied by means of seeds (sexual propagation) or organs other than seeds (asexual or vegetative propagation). These organs are usually stems, leaves or roots. 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 vegetative 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, which could overcome disadvantages of propagation by seeds or vegetative organs, was found commercially successful in the case of orchids. Subsequently, the method has been perfected for many other plants (Annexure A). The method (also known as micro-propagation) involves the culture of whole organism from cells or tissues or plant parts in glass (in vitro) on a defined medium under germ free conditions (sterile or aseptic), whereas conventional method of vegetative propagation (macro-propagation) involves culture of parts into whole organisms in natural conditions (in vitro).

2. Major advantages of Tissue-Culture:

A large number of true to the type plants could be propagated within a short time and space and that too throughout the year. For example, it may be possible to propagate 2-4 lakhs of Tissue Cultured Plants (TCP) from a single bush of rose against 10 to 15 plants by vegetative means. Also, it may take about 2-4 months to produce healthy planting materials by tissue culture means, whereas a minimum of 6-8 months is required for most species by the latest method of vegetative propagation.

Tissue-culture could be a useful way of circumventing or eliminating disease which can accrue in stock 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.

The method saves space and energy. For example, 2500m2 of heated green house can be replaced by a climatised room of 10m2. The flexibility of nurseries can be improved. 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. Tissue culture can be utilised for breeding new varieties, preservation of germplasm and in vitro synthesis of metabolites

3. Commercial prospect

Propagation by tissue-culture offers good commercial prospect in ornamental plants, vegetables and also fruit plants, where value of the products is high. The technique has reportedly been successful in more than 100 species of plants. It has been estimated that more than 350 million TCPs are being produced annually through tissue culture.

In India there are at least ten commercial organisations, which have developed technical competence for tissue culture with or without foreign tie-ups. The present installed capacity is about 50 million TCPs and the export is of the order of Rs. 5 crores. The working group appointed by the Ministry of Commerce has proposed an export target of Rs. 30 crores i.e. about 60 million TCPs over a five year period as against the present production of 8-10 million TCPs. Tissue culture method of propagation is highly labour intensive, 55-60% of the cost is on account of labour. India's potential for export of tissue culture plants is rated very high because of abundant and cheap labour.

The domestic market for TCPs, though nascent at present, is likely to develop, since tissue culture method of propagation can multiply an elite plant very rapidly. It is well known that one of the major constraints of horticultural development in our country is inadequate availability of quality planting materials.

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 by conventional means.

4. Tissue-Culture Technology

The details of technology are given in the Annexure O.

5. Objective of Tissue-Culture Project

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

6. Requirements of Tissue-Culture Projects

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, given in the Annexure- O 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 staffs 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. Inside air of laboratory should be free from dust particles (Annexure D).

6.5 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 specie. Sugar is the source of energy since tissues and shoots, while in the laboratory do not normally photosynthesize. Other

nutrients perform their usual structural, functional and catalytic role. The agar is added to solidify the medium. Commercial tissue culture received major boost with the development of an improved medium in early sixties by Murashige and Skoog (**Annexure B).**

6.6 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:

Computerization 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.

vii) Air Conditioners with Stabilizers:

Maintenance of desired temperatures 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 primodia.

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 maximise utilisation 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 bottoms 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 Typewriters etc.

6.7 Green House

xiv) Tube lights:

Fluorescent tube lights are mounted on the bottoms 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, Typewriters etc.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 1.25 million TCPs per year. It has been estimated that, to produce 1.25 million TCPs, a laboratory of 5000 sq. ft. would be required. A green house facility of 5000 sq. ft. for maintenance of mother plants and Hardening tissue cultured plants would be helpful.

9. Estimated Cost.

The estimated cost for production of 1.25 million plants is indicated below.

The recurring expenditure of only 1st year may be capitalized. Thus, the total cost may amount to Rs. 207.74 lakhs.

The estimated cost does not include cost of land. For 100% export-oriented units, land cost, may be included in the above estimate depending on merit of each case subject to a limit.

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

	Particulars	Rs lakhs
A	**FIXED COST**	

i	Tissue culture laboratory including green house and green house		52.85
	equipments (Annexure E)		
ii	Laboratory Equipments (Annexure F)		42.36
iii	Furniture, Fixtures and Office Equipments (Annexure H)		7.41
iv	Water supply system (Annexure G)		2.00
v	Training		1.50
vi	Consultancy / Know-how fees		2.00
			56.23
B)	Contingency		5.623
			169.97

C)	Recurring Cost (Annexure K)		
	Year 1		37.77
	Year 2		50.086
	Year 3		57.62
D)	Capital Cost (Rs.169.97 + Rs.37.77 Lakhs)		207.74

10. Projected Benefit.

Of the total production of 12,50,000 TCPs, about 10,00,000 TCPs (80%) may be expected to be of exportable quality.

A net average price of Rs. 12.00/unit (net of expenses abroad on transport, commission, etc.) may be considered as reasonable. The setting up of laboratory and other facilities and Standardisation of the tissue-culture protocol may take about one year. However, 20,000 TCPs may be produced in the 1st and 2nd year for distribution as free samples. Therefore, no income has been projected in the 1st year. Thus the benefit (Annexure L) is projected as under:

11. Market Development

The commercial prospect of tissue culture has been mentioned under paragraph 3. 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 Viability

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

NPW at 15% DF -- Rs. 114.68 lakhs

BCR " " -- Rs. 1.92 lakhs

IRR " " -- 27%

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. Repayment

The interest payment will start from 2nd year and the repayment of principal from the 4th year. The entire loan with interest may be repayable over a period of 8 years.

(**Annexure N).**

Annexure A

Plant Species Reported to respond to propagarion by Tissue Culture

A. Ornamental plants

Philodendron, Gladiolus, Diffenbachia, Lily, Monstera, Kalanchoe Maranta, Pentunia, Alocasia, Narcissus, Aloe vera, Rose, Fern, Mimosa, Ficus benjamina, Gypsophila, F. elastica, Aster, F. lyrata, Aglaonema, Ficus robusta, Hydrangea, Ficus mini, Amarylis, F. compacta, Nerine, F. foliole, Tulip, Iris, Nephrolepis, Freesia Calathea, Hyacinth, Heliconia, Anemone, Syngonium, Begonia, Dracaena, Eucharis, Peperomia, Caladium, Euphorbia, Carnation, Pelargonium, Chrysanthemum, Platycerium, Gerbera, Pteris, Saintpaulia, Davallia, Streptocarpus, Osmunda, Anthurium, Mammillaria, Aechmea, Christmas Cacti, Cyclamen, Easter Cacti, Kalanchoe, Agapanthus, Episcia, Asparagus, Spathiphyllum, Gloxinia, Alstroemeria, Guzmania, Hamamelis, Cordyline, Hemerocallis, Schefflera, Dendrobium, Liriope, Cymbidium, Strelitzia, Cattleya, Nandina, Odontoglossum, Rhododendron, Phalaenopsis, Bougainvillea, Vanda, Buddelia, Weigela, Magnolia, Ribes, Deutzia, Epidendrum, Crocsmia, Forsythia

B. Vegetables

Onion, Asparagus, Brassica, Tomato, Chicory, Capsicum etc.

C. Fruit plants

Banana, Strawberry, Apple, Pear, Cherry, Citrus, Rubus, Gooseberry, Grapes, Papaya, Pineapple, Ananas etc.

D. Forest species

Poplar, Eucalyptus, Bamboo, Pinus, Cupressus, Thuja, Sequoia, Ulmus, Spiraea

Betula, Salix, Ilex, Fagus, Picea etc.

E. Others

Coffee, Cardamom, Ginger, Turmeric, Vanilla, Hops, Oil Palm etc

(a) Assuming gross area of 140 ft. x 100 ft. i.e. 14,000 sq. ft.

(b) Includes cost of electrical wiring, plumbing, architects fees, fees to statutory authorities, Electricity Boards etc.

(c) For details of the estimates, vide model on carnation project.

N.B.: Estimated costs are to be supported by designs, rate analysis and quotations wherever necessary.

ANNEXURE-D
TISSUE CULTURE LABORATORY
CIVIL STRUCTURES

Year	**Total Production**	**Sale (No.)**	**Gross Income**

	(No.)		**(Rs. lakhs)**
1	10,000	NIL	NIL
2	1,000,000	750,000	90
3	1,250,000	1,000,000	120
A.	**Clean Area**	Floor Area	
		(Sq.ft.)	
	1. Media Store and Production Control	200	
	2. Post Autoclave Area	150	
	3. Culture Transfer Room	300	
	4. Growth Rooms		
	(i)	250	
	(ii)	250	
	(iii)	250	
	5. Change Area	100	
B.	**Semi-Clean Area**		
	6. Legwash	100	
	7. Laboratory / Media	400	
	Preparation/Auto Clave		
	8. Wash Area		
	(i) Bottle	200	
	(ii) Plant	200	
	9. Store (consumables)	250	
C.	**Service Area**		
	10. Office Lobby, corridor	550	

	11. Scientist Room	200	
	12. Computer Room	100	
	13. Genset Room	150	
	14. Canteen	200	
	15. Toilet	150	
	Total		
	Covered Area (approx.)		

ANNEXURE-E
CIVIL STRUCTURES

Sl. No	**Particulars**	**Area**	**Rate (Rs.)**	**Amount (Rs.)**
1	Boundary Wall (a)	4800	500	240,000.00
2	Laboratory (b)	50000	800	4,000,000.00
3	Auxilary Structure	500	700	350,000.00
4	Polyhouse			
	(i) Mother plant area (c)	20000	100	200,000.00
	(ii) Hardening area (c) for Plantlets	30000	10	200000
	(iii) Polytunnels-25 nos each of 40 sq.ft	10000	10	5,000.00

	(iv) Wirenet for polyhouse walls	3600	20	72,000.00
	(v) Sunshade net	20000	5	72,000.00
	(vi) Exhaust Fans	4	10000	72,000.00
	(vii) Trays	400	200	72,000.00
	(viii) Thermometer Hygrometer etc			2000
				5,285,000.00
				52.85

ANNEXURE-F
LABORATORY
EQUIPMENTS

Sl.No.(1)	**Particulars (2)**	**No. (3)**	**Rate Rs./pc (4)**	**(Rs.lakhs)(5)**
1	Autoclave	2	250,000	5.00
2	Balances	2	50,000	1.00
3	pH meter	2	10,000	0.20
4	Laminar airflow	2	150,000	3.00
5	Distillation set	2	40,000	0.80
6	Computer System	2	50,000	1.00
7	Air-conditioners :			

	a) 1.0 tonnes	6	20,000	1.20
	b) 1.5 tonnes (2 standby)	8	35,000	1.20
	(For about 1500 sq.ft. area, with two standby)			
8	Microscopes		40,000	0.00
9	Bottle washing unit	1	350,000	3.50
10	Media cooking unit	1	150,000	1.50
11	Growth room racks (6 racks/room)	18	10,000	1.80
12	Trays (4 trays/shelve)	600	200	1.20
13	Trolleys	8	2,000	0.16
14	Diesel Genset (62.5 KVA)	1	313,000	3.13
15	Dissecting Kits and Inoculation instruments		40,000	0.00
16	Refrigerator	1	20,000	0.20
17	Air filters	2	20,000	0.40
18	Oven	1	20,000	0.20
19	Rotary Shaker	2	30,000	0.60
20	Bottles	30,000	4	1.20
21	Lab clothes	-	30,000	3.00
22	Washing machine	1	25,000	0.25
23	Incinerator	1	30,000	0.30
24	Fire fighting equipment	-	35,000	3.50
25	Stabilizers (10)	-	30,000	3.00

26	Miscellaneous Glassware	-	40,000	4.00
27	Tubelights for growth rooms	600	170	1.02
				42.36

ANNEXURE-G

FURNITURE FIXTURES AND OFFICE EQUIPMENTS

		Rs	Rs
1	STW - 2"	10,000	
2	Overhead Tank (1000 litres)	10,000	
3	Pumpset (3 HP)	10,000	
4	Pump House	15,000	
5	Mist system for Green house	50,000	
			95,000

Annexure-H			
1	Tables for GM and Assistant Managers	40,000	
2	Clerk	5,000	
3	Lab. Tables	40,000	
4	Chairs and Sofa set (visitors tables)	45,000	

5	Cupboard	25,000	
6	Lab. racks	15,000	
7	Miscellaneous	10,000	180,000
8	Tube light for offices, lobby etc., (25)	5,000	
9	Fans (b)	10,000	
10	Fax Machine	10,000	
11	Telephone	1,000	
12	Pick up Van	440,000	466,000
	Total		741,000

Annexure I			
Overheads			
A.	**Salary**		Salary/year (Rs.)
1	General Manager 1 (Scientist-in-charge)		360,000
2	Assistant Managers (Rs.15,000 p.m.)		540,000
	a. Laboratory - 1		
	b. Greenhouse - 1		
	c. Marketing and office - 1		
3	Operators (Rs.5,000 p.m.)	16	960,000
4	Helpers (Rs.3,000 p.m.)	10	360,000
5	Clerk-cum-Typist	1	60,000
6	Guards	3	144,000
7	Driver	1	60,000

8	Mechanic / Overseer	1	36,000
9	Contingencies		12,000
			2,532,000

Annexure J

Power requirement

1. Tube lights for growth room

Assuming 32 tube lights / rack (4 in each shelve, total number of tube lights for 18 racks in 3 rooms is 576, say 600.

Therefore 600x40 Watt x 18 hr. 365 days = 1,57,680 k.Watt.hr. Say 1,58,000 units.

2. Air conditioners

A.C. 1 tonne, 6x1.5 KW = 9 KW

A.C. 1.5 tonnes 6x2.5 KW = 15 KW

24 KW

Therefore 24 KW x 24 hrs x 365 days = 2,10,240 KW hr. = 2,10,000 units

3. Exhaust Fans for Green house

4 (no. of fans, 24") x 0.5 KW x 12 hr. x 365 days = 8760 units, say 9000 units.

4. General Lighting

25 (No. of tube lights x 40 x 12 hrs. x 300 days) = 3600 units

Fans 6 x 60 x 12 hrs. x 300 = 1300 units

Total = 4900 units, Say 5000 units

Total 1.58 + 2.10 + 0.09 + 0.05 lakh units = 3.82 lakh units

Consumption for misc. laboratory equipments, 0.68 lakh units (on lumpsum basis). Therefore, total consumption may be about 4.50 lakh units. Assuming electricity tariff of Rs. 1.00/unit for agricultural purposes.

Estimated Cost = Rs. 4.50 lakhs.

Annexure K

RECURRING EXPENDITURES

(Rs. lakhs)

ANNEXURE-K
RECURRING EXPENSES

S.No.	**Item**	**Y E A R S**		
		1	**2**	**3 onwards**
i	Salaries and wages	25.32	26.586	27.915
ii	Laboratory consumables	0.5	1	1
iii	Greenhouse rooting media	0.3	0.6	0.6
iv	Mother plant	0.2	0.2	0.2
v	Power	1.5	7	9
vi	Fuel	0.5	1	1.2
vii	Packaging	-	1.5	2
viii	Air freight	-	1	1.5
ix	Administrative			
	a. Printing & Stationery	0.15	0.3	0.3
	b. Postal, Telephone Telex	0.5	1	1

	c. Travels	1	1	1
	d. Books and periodicals	0.5	0.5	0.5
x	Market Development			
	a. Foreign visits for marketing contract	2	2	3
	b. Publicity abroad	1	1	1
	c. Distribution of free samples	1	1	1
xi	Repairs and Maintenance(including replacement of polythylene)	-	1	3
xii	Insurance	1.2	1.2	1.2
xiii	Breakage of Glasswares and bottles(10% p.a.)	0.1	0.2	0.2
xiv	Contingency (includes replacement of roofing material, LDPE for greenhouse)	2	2	2
		37.77	**50.09**	**57.615**

Note: 1) Interest in the I year is paid on the III year since one year grace period for payment of interest

2) Grace period for repayment of principal is 3 years

ANNEXURE-L

estimated production			
Sl.No.	**Item**	**Details**	
1	No of work stations in Laminar Flow cabinets (2)	16 (in two shifts)	
2	Total Floor spacein Growth Rooms	750 sq.ft.	

3	Total space in 18 Racks each with 8 shelves	8x18x18	
	(each shelve 18 sq.ft.)	= 2592 sq.ft.	
4	Total No. of culture bottles (About 200 bottles/shelve)	200x8x18	
		= 28,800	
5	No. of bottles to be used for shoot multiplication cycle	25,000	
	(Assumption 86% capacity utilisation)		
6	No. of shoots to be produced/multiplication cycle(Assuming multiplication ratio as 5)	125,000	
7	Total production of shoots per year assuming 10	12,50,000	
	multiplication cycles		
8	Total exportable production (Assuming 80% of Total)	1,000,000	

Annexure-M

ANNEXURE-M

FINANCIAL ANALYSIS

S.No	Item	Y E A R S							
		1	2	3	4	5	6	7	8
1	Fixed Cost	169.97							
2	Recurring Cost	37.77	50.09	57.62	57.62	57.62	57.62	57.62	57.62

3	Total Cost	207.74	50.09	57.62	57.62	57.62	57.62	57.62	57.62
4	Benefit		90.00	120.00	120.00	120.00	120.00	120.00	120.00
		-207.74	39.91	62.38	62.38	62.38	62.38	62.38	62.38
		0.87	0.76	0.66	0.57	0.50	0.43	0.38	0.33
5	PW of Cost at 15% DF	180.65	37.87	37.88	32.94	28.64	24.91	21.66	18.83
6	PW of Benefit at 15% DF		68.05	78.90	68.61	59.66	51.88	45.11	39.23
	NPW		30.18	41.02	35.67	31.02	26.97	23.45	20.39
	BCR		1.80	2.08	2.08	2.08	2.08	2.08	2.08
	BCR	1.92							
	IRR	0.27							

ANNEXURE-M

FINANCIAL ANALYSIS

S.No	Item	9	10	11	12	13	14	15	
1	Fixed Cost	57.62	57.62	57.62	57.62	57.62	57.62	57.62	
2	Recurring Cost	57.62	57.62	57.62	57.62	57.62	57.62	57.62	
3	Total Cost	120.00	120.00	120.00	120.00	120.00	120.00	120.00	
4	Benefit	62.38	62.38	62.38	62.38	62.38	62.38	62.38	
		0.28	0.25	0.21	0.19	0.16	0.14	0.12	
		16.38	14.24	12.38	10.77	9.36	8.14	7.08	281.
5	PW of Cost at 15% DF								
		34.11	29.66	25.79	22.43	19.50	16.96	14.75	574.
6	PW of Benefit at 15% DF								
		17.73	15.42	13.41	11.66	10.14	8.82	7.67	322.
	NPW	2.08	2.08	2.08	2.08	2.08	2.08	2.08	207.
	BCR								
	BCR								114.
	IRR								

Sales-
Benefits

BENEFITS		TCP	Rate	Value
SALES			Rs/TCP	Rs lakhs
Year	1	0		
	2	750000	12	90.00
	3	1000000	12	120.00

	4	1000000	12	120.00
	5	1000000	12	120.00

ANNEXURE-N							
REPAYMENT SCHEDULE							
Year	**Incremental benefit**	**Bank Loan Outstanding**	**Repayment**			**Outstanding at the end of the year**	**Surplus**
			Interest at 12%	Principal	Total outgo		
1	-207.74	155	18.6	0	18.6	155	-207.74
2	39.91	155	18.60	0	18.6	155	21.31
3	62.38	155	6.60	26	32.6	129	29.78
4	62.38	129	6.60	26	32.6	103	29.78
5	62.38	103	5.52	26	31.52	77	30.86
6	62.38	77	4.32	26	30.32	51	32.06
7	62.38	51	3.00	26	29	25	33.38
8	62.38	25	1.44	25	26.44	0	35.94

Annexure O

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 organism. 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.

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 Standardised through trial and error and ultimately it should be repeatable and reliable **(Annexure C).**

1 Stages of Tissue Culture

The stages involved in propagation by tissue culture are dividend into five. A general account of these stages is outlined below.

A 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. Activity growing (shoot tips), juvenile (seedlings) or rejuvenated (suckers) tissues are preferred.

The commercial tissue culture labs commonly use tips of apical or lateral shoots, which contain meristems. Meristems are made up of cells dividing actively in an organised 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.

The quantity of explant required for propagation by tissue culture is very small. For example, 2 mm. thick petiole sections from African violet (a flowering herb) could yield 20,000 plantlets per petiole (basal portion of leaf). Foreign/local collaborators with established business may agree to supply explant free of cost.

b. Establishment of Germfree (aseptic/sterile) culture

Excised part of plant is surfaced sterilized and transferred to sterile nutrient medium contained in glass vessel. On an average, about 50 cc. nutrient medium 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 artificial environment, initiation of new growth will take place after a week or so. Thus, germ-free culture is established.

c. 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. Hormones (cytokinins) are used to induce multiple branching. This is the most common method followed in commercial units. However, 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 also can 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 (a) and (b) 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.

The formation of callus can be induced by selecting proper tissue and culture medium. 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 o tissue, genotpye, 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.

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

Young axillary or adventitious shoots are finally separated form 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.

e. Establishment in the natural environment

The most critical stage of the propagation by tissue culture is the establishment of the plantlets into 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 normal over 3-4 weeks,

- And gradual increase in light intensity.

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

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 200 - 300 C depending on species of plants. Tropical plants may require higher temperature than temperature plants.

3. 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 are 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 of disease. Meristem is usually free from disease. Surface sterilisation 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 1200 C for 15 minutes. The laboratory should be cleaned with disinfectants. Workers should wash their hands and feet with disinfectants before entering the laboratory. They should put on sterilised clothes. 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 air flow 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.