APPLICATION OF LOW COST TISSUE CULTURE TECHNIQUES FOR FLORICULTURE

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மனிதவலு மற்றும் வேலைவாய்ப்பு திணைக்களம்
Department of Manpower and Employment

Department of Manpower and Employment

Cases Study - 2022

1. Introduction

In the global scenario annual floricultural traded value is around 50 billion US $. Netherland is the main importer and, as well as main exporter. It is a small country with compared to Sri Lanka but, that country import, then value add and re-export in large volumes of diverse floricultural products especially to European and American markets. Sri Lankan contribution to the global floricultural market is 0.2% (Department of National Planning; Project Recommendation Letter, 2016). The export value of floriculture in 2021 was US$ Million 16.19 and the share of total export was 0.13 percent. (https://www.srilankabusiness.com/floriculture/floriculture-export-performance.html). As a tropical country our climate is very conducive to development of floricultural sector. It's diversity is very high and that also can be used to develop new varieties in commercial importance. There is greater scarcity in mother plants in commercially high demanded species, clones. Even local demand cannot be met by local production. On the other hand Sri Lanka is importing huge amount of floricultural plants especially from Thailand (Discussion on Micropropagation – Based Floriculture Entrepreneur Development program). That depletes Sri Lankan foreign reserves and various invasive virulent pest and diseases can come to the country that can ruin entire floriculture industry. If

researcher introduces new species or varieties, there is a limitation in multiplying using conventional methods like various vegetative cuttings, seed culture in order to fulfill commercial demand. If seed culture is used, commercially important identical progeny may not result. Therefore, the conventional methods have limitations in commercial level mass production. Hence, it has to move to in-vitro propagation techniques which are available in amarelle of Tissue culture as micro propagation methods.

There are two methods of tissue culture techniques available in Sri Lanka at present, first one is widely practiced Traditional Method (Large scale, high capital, input and energy usage method) and other method is an Alternative Low Cost Method which was invented by the faculty members of Department of Crop Science in Faculty of Agriculture, University of Peradeniya. Conventional production process is complex and that needs high amount of electrical energy to sterilization of glassware and culture media, culture establishment process and maintenance of culture room. In low cost method mainly sterilization is done by using proven alternative method which have patent. Lab procedures are simple in that method and also lab lay out is simple and small. Therefore energy requirement is lower than traditional laboratory which have same production capacity.

2. Objectives of the Study

- To provide information on techniques and cost estimation of tissue culture lab for creates new job opportunities.
- To make recommendation for how to maintain a tissue culture lab to families who wish to commence entrepreneurship to upgrade their income level over the poverty line.

3. Importance of the Tissue Culture

Using Micropropagation techniques can produce large amount of identical plants using small space and time in a low coat manner (Plants 15,000 – 100,000).

4. Introduction to Tissue Culture Development

Plant cells possess the ability to develop as a complete Plant. This potential is called as Totipotency. Based on this concept several research attempts have been done by the scientists to develop viable method/technique. Above intensive scientific research lead to development of present plant tissue culture techniques. Initial technique is called as conventional techniques and later several low cost tissue culture techniques have been developed.

Several plant parts, seeds, pollens, ovules, nodes, leaf parts, apical buds can be used as source of material for mass scale tissue culture; that is scientifically called as X- Plant material.

Growing of plants, plant parts, organs or cells in aseptic environment under controlled condition using artificial medium is defined as tissue culture in general. Developed several tissue culture techniques examples are given below: (නාගහවත්ත, එස්.එම්; ශිරාණි, ඩී.ඒ.; කරුණානන්ද ඩී.පී.,2016)

1. Micropropagation
2. Pollen culture
3. Ovule culture
4. Nodal culture
5. Leaf culture
6 seed culture
7. Embryo culture
8. Callus culture

5. Basic Requirements of Conventional Tissue Culture Laboratory

Since the tissue culture practiced under the aseptic condition, every step has to be preserve aseptic conditions. The lab layout should be designated as it is.

5.1. Washing Area

In this are material which are taken from outside is cleaned and prepared as required to the lab procedures. The glassware also cleaned in this apartment. Aseptic conditions are low in this area.

5.2. Media Preparation Area

.This is second apartment. Heat generation is occurred in this area due heat based sterilization. Auto clave are operated for sterilization media preparation from stock solution is done.

5.3. Transfer Room

In this aseptic condition are highly essential, Laminar floor cabinet are used. This area is maintained under the Air Condition. Callus establishment and subculture are done here.

5.4. Culture Room

Sub cultured bottles are grown accordingly to it's culture cycle. This area is kept air conditioned. Culture bottles are kept in racks and artificial light should be supplied as per the crop requirement.

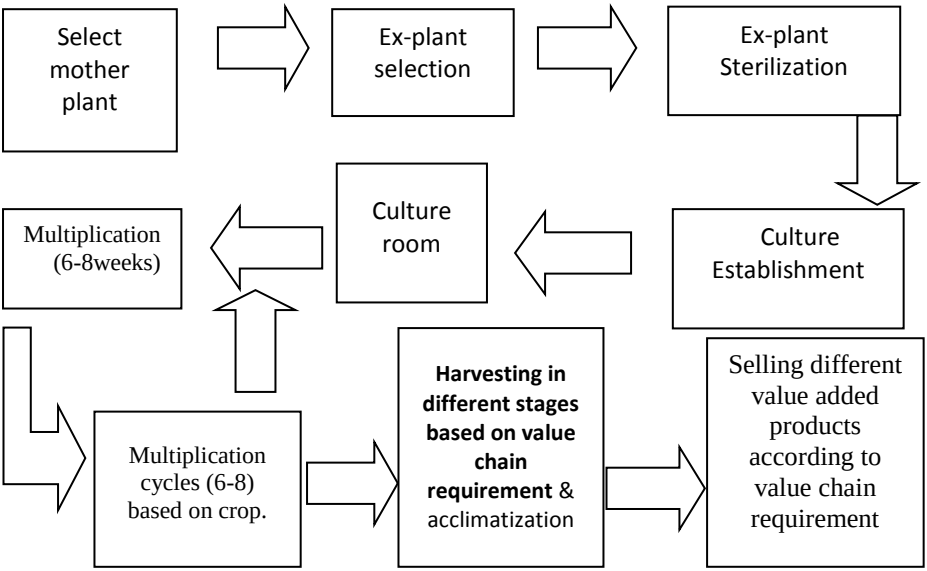
Matured bottles removed periodically and those are send to acclimatization process. Conventional tissue culture laboratory needs approximately LKR 3 – 3.5 Mn.

5.5 Acclimatization Process

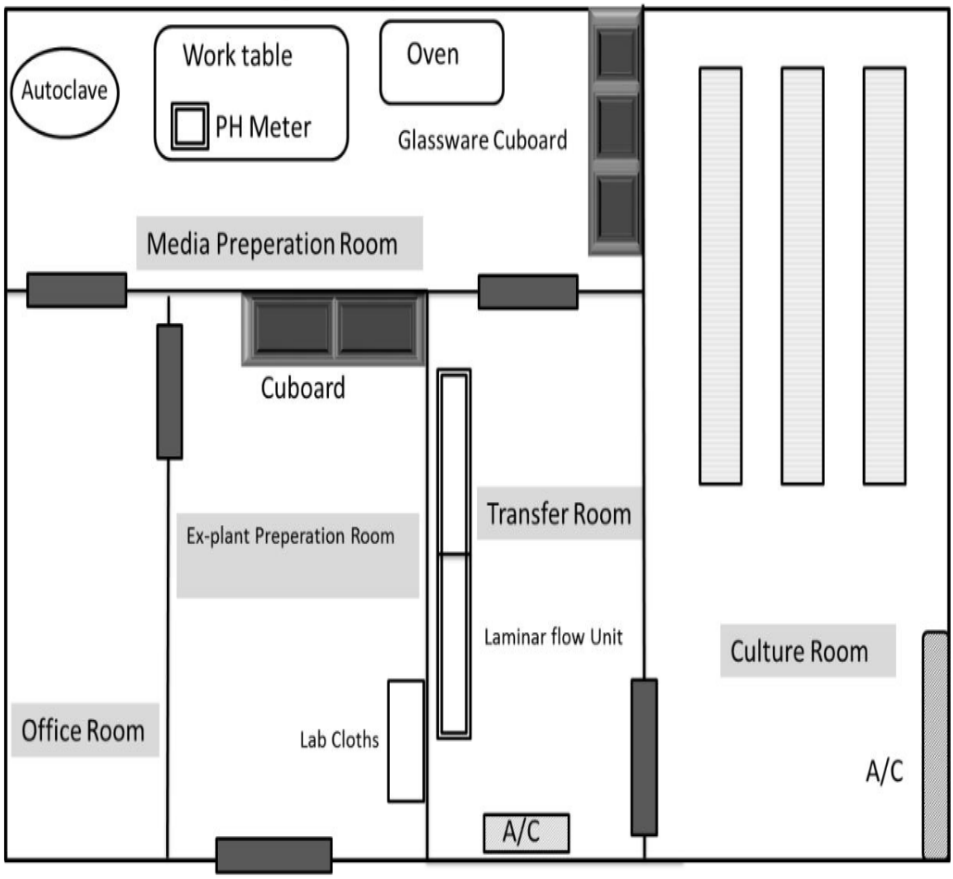
Plant lets in the culture bottles in the culture room cannot with stand sunlight. Chiteen cover is not developed in initial stages. Therefore plant can be damaged from direct sunlight. Hence, gradual preparation to hard condition is called as acclimatization is needed. That process has to be performed before sending to the market. After acclimatization can grow further in plant house or can sell directly.

5.6 Production process

Exhibit 1 : Production Process



5.7 Exhibit 2 :Conventional Lab Diagram – Top View



(Average size is Sq. Feet 1,400 – 1,500)

5.6 Required Equipment for Conventional Tissue Culture Lab

1. Auto Clave Unit

2. Laminar Air Flow Cabinet

3. Water Distillation Unit

4. Hot Air Oven

5. Refrigerator

6. Mechanical Shaker

7. Microwave Oven

8. Electronic Balance

9. Analytical Balance

10. Ph Meter

11. Stirrer with Hotplate

12. Racks

13. Glassware

14. Air Conditioner

6. Recommendation/ Selection of Method

Selection of Conventional Method or Alternative Low Cost Method depends on several factors. Investment ability can be considered as a first factor. Next standards set by the main buyers, scale of production, ability of investment, available space, available technical and consultancy support are considers as main determining factors. For house hold level small operations alternative low cost method is recommended because it's operations are simple and energy cost is minimum compared to of Conventional Method (Conventional method is described in the Section 5).

7. Land Requirement:

1. Conventional Laboratory – 30 x 50 sq. ft. (1400 – 1500 sq.ft.)
2. Alternative Low Cost Laboratory -20 x 12 sq.ft. (200 sq.ft.)

8. Scale-up : Conventional lab can be designed in to large capacity at the construction stage and that can be partitioned (specially culture room) according to the market requirement. When the demand is getting higher the partitioned stagnated space can be used to production. Accordingly the lab can be scaled up according to the market requirement.

For the low cost tissue culture lab construction cost, space and the investment is comparatively low. Therefore, another lab or laboratory units can be constructed according to the production and market requirement easily.

9. Registration Requirements:

1. Seed Handler Registration Certificate can be obtained from the Department of Agriculture.

SA/PAL/14590

Registration No

Seed Act No. 22 of 2003

Section III 8

Seed Handler Registration Certificate

This is to certify that

.....

.....

.....

has been registered as a

Planting Material Producer & Merchant of

.....Flowering & Ornamental Plants.....

From ..... to.....

Seed Certification Service

Seed Certification & Plant Protection Centre

Department of Agriculture

.....

Director

Seed Certification and Plant Protection Center

Gannoruwa, Peradeniya, Sri Lanka

2. To manage commercial venture, owner should obtain the business registration from the relevant Divisional Secretariat.

10. Alternative Low Cost Tissue Culture Techniques

(10% Sodium Hypho-cloride solution based (Bleach) Techniques)

10.1. Innovation

1. Introduction of CSUP solution for culture media and glassware sterilization instead of high energy consuming Autoclave. This method is patented one (10% Bleach).
2. Use of glass box and/or glow bow instead of High cost Laminar Flow. (Locally manufactured micro filter unites also available at a very low cost instead of imported laminar flows.)
3. Simplified tissue culture laboratory arrangement, which save energy and movements.
4. Household pressure cookers can be used to prepare sterile water.
5. House hold burners can be used.

10. 2.Technical Feasibility

1. Introduction of CSUP solution for culture media and glassware sterilization instead of Autoclave is proven by research.(2006)
2. This CSUP/NaOCl method has been proven by research at faculty of Agriculture, University of Peradeniya and research paper has been published by the researcher. Now this method is a patented procedure.

3. Commercial level Application of this research finding can be observed in *Dodangolla research farm* premises of University of Peradeniya.

10.3. Project Duration : 2- 5 Years

To commence full production lab takes 2 years from the initiation because 8 - 10 micropropagation cycles should be performed to obtain maximum production capacity to be cost effective in operation. This time can be reduced by selected disease free culture bottles from other laboratories and start multiplication from them.

10.4. Project beneficiaries:

a. Buyers from the Social Media Networks

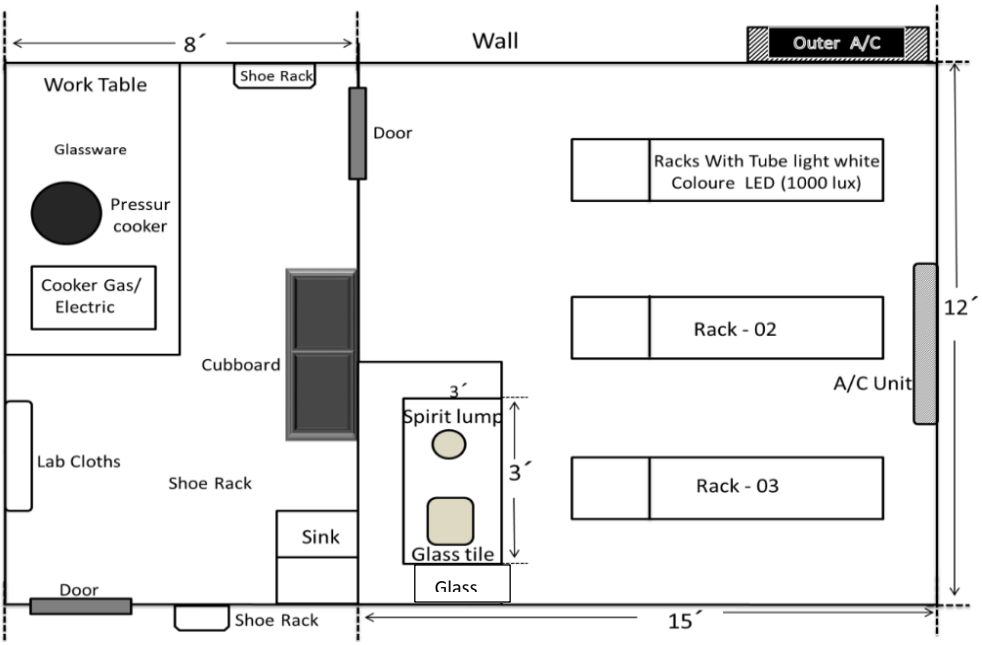
b. 5-10 conventional nursery owners in the divisional secretariat area and cut flower exporters in the area as buyers of produced plants.

c. Unemployed youth as lab assistants (2) and nursery workers (3)

d. Chemical, lab equipment and nursery items supplying small scale entrepreneurs (3)

e. Buyers, resellers and final consumers in the floriculture product value chain due to guaranteed quality of the product at a affordable market price.

Exhibit 3 : Sample Diagram of Low Cost Lab Unit - Top View



10. 5. Important Factors in preparing and managing low cost Tissue Culture Lab unit

1. Preparation of lab unit/ Room

I. Walls, Roof, and windows should be sealed in order to prevent dust, prevent leakages of air conditioned air and light.

II. Window margins should be sealed with Silicon Gum

III. Room Celling also should be sealed with silicon gum to prevent leakages of dust and air.

IV. Fungal proof and washable paint should be applied to inside walls of the lab unit.

V. The door should be constructed as much as air sealed manner in order to prevent air movement, to prevent possible contamination and to prevent inside air conditioned air losses.

VI. It is preferable to select top surface in concrete (it is not compulsory)

VII. Racks should be constructed in standard size.

2. Lab unite inner wall scartin and Floor

Scartin upper surface should be round shaped in order to minimize contamination and cemented floor is preferred it is also to minimize the contamination.

3. Racks

Racks should be prepared according to the sizes given below.

From floor to first rack bed one and half feet distances have to be kept. Width of the rack generally Two feet and normally four rack beds are available in one rack unit. The length of individual

rack should be determined according to the space available in the lab.

Enough spacing should be kept in-between racks and walls also in order to facilitate easy handling and rack disinfection procedures.

Low cost LED Bulbs can be used in lighting of the racks; generally four feet and 18 watt LED bulbs are commercially used. Timer Switch should be installed to the to the lighting system of the lab unit in order to regulate light duration for cultures. It is advisable to install separate Electrical switches to on or off separate rack or necessary rack beds to save the electricity.

4. It is advisable to install an Air condition unit in the initial stage because the heat generated should be removed from the system.

5. Lab Disinfection and Sterilization (Pest and Disease Controlling)

I. Before initiation lab operations the floor, racks, rack beds and all the equipment which are kept inside the lab have to be wiped with a disinfectant like Dettol or Savlon.

II. After that racks should be wiped with 95% Ethyl Alcohol or surgical sprit or 2% Clorox solution

III. Then before commencement of lab operation first time 95% ethyl alcohol or surgical spirit (Isopropyl Alcohol) should be sprayed inside the lab and lab should be kept adequate time period for proper sterilization.

IV. In lab sterilization Phomalin also used, but it is highly toxic to the human. Therefore use of that chemical is done under the qualified technical supervision only. After applying this chemical normally one week the lab should be kept closed without entering. This chemical is only used instances where any pathogen is speeding vigorously and that cannot be controlled by other chemicals. Also this can be used in the starting point of the laboratory.

V. Bactericide is not recommended for use in the Lab for controlling Bacteria because wild bacteria variant can generate resistance to the bactericide which used for human treatment. Therefore all the culture bottle and equipment sterilize using wet heat (Keep in 121 Celsius for 21 minutes under 15 psi)

6. When entering and using the lab have to use clean white colour Lab Cloak and Clean shoes.

7. Within the lab unit other than the racks, Glass box should be installed for culture establishment and culture transfer procedures; this is used instead of laminar flow cabinet. Its dimensions are shown is this diagram.

8. **Required Equipment**

Small Refrigerator

Air Conditioner (12,000 BTU)

Pressure Cooker (12 L)

Electric Oven, Gas Cooker

Pyrex Glassware

Glass Box

Chemical : Murushige & Scooch (MS) Medium

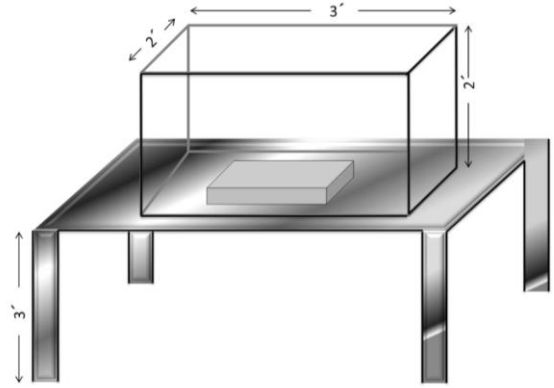
Culture Racks - 02 (4 Beds)

18w LED Tube Light 32 Nos

Measuring Cylinders, Pepets, Funnels/ Beakers, Hotplate

Bleach Commercial

Exhibit 4 : Glass Box Dimensions



Glass Box for Low Cost Lab units

9. Before commencement of the work this Glass box should be sterilized. That should be wiped with 70% surgical sprit. The glass pad and hands also should be sterilized using same chemical above. After this procedure glass box have to be kept approximately 15 minutes without inserting Spirit lamp, (It is flammable with surgical spirit)

10. In adjacent to the lab cleaning and media preparation area should be prepared. Balance,PH meter,Pressure Cooker, Glassware, microwave oven, refrigerator, chemicals can be kept .The wash basin is also essential.

11. Glassware Sterilization

The success of this low cost tissue culture technique is entirely relay on glass ware sterilization and the concentration of Clorox .(Bleach)

Selected cleaned jam bottles or test tubes should be washed with 10% bleach after that glassware are not wiped out and should be kept upside down arrangement on a cleaned tray. In this procedure face masks and glows should be wore.

12. After that culture media is prepared.

Generally MS medium is used; other low cost media also can be used according to the crop. Those solutions come as stock solutions. Trace elements, basic elements, iron and vitamins are comes as stock solutions .in preparation of medium given volumes and prescribed sequence of mixing should be followed. Sugar, gelatin, mio-inositol, hormones also should be add accordingly. PH should be adjusted. For media preparation distilled water can be used. After adding required ingredients for the medium it should be boiled.

After that media should be filled to the previously sterilized containers (Jam bottles / test tubes) and those should be closed

with cellophane sheet and labeled. Above bottles and tubes should transfer to glass box apprator for culture establishment or transfer.

13. Disease Control

At the starting point of the lab, all the racks flow and walls wipe with Savalon or the Dettol as the preliminary disease control method. Secondly, racks are wiped with Clorex 10 percent solution. After that spraying of Ethyl Alcohol and wiping with Ethyl Alcohol is considered as disease control method. When some disease is spreading in a severe manner Formalin treatment is recommended.

All the disease infected glass bottles should be serialize using a Pressure cooker in a 121 Celsius for 21 Minutes under 15 psi. Disease infected containers can also be sterilize by using concentrated Clorex.

Root fungal Disease - Captan, Homay, Thiram, Mancozeb, Maneb, Zineb, Copper Fungicide, Copper Oxychoride

14. Fertilizer :

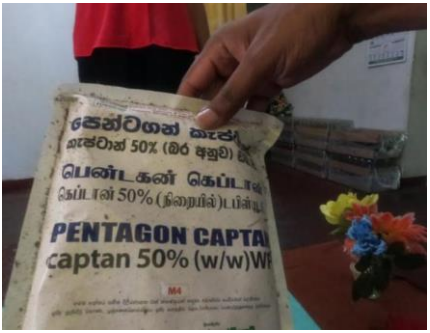
The plantlets with in the culture bottles do not require additional fertilizer from outside the culture bottle which contains growth medium supply required morticians with in the laboratory period.

Therefore no need of fertilizing to the plant which are in the laboratory (Multiplication cycle)

After sending to the nurseries plants need high posperous and potassium to trigger root growth (Posperous high). Then plans are supplied balance NPK with micro nutrition's for vegetative growth (Balance NPK). Then in the flowering period plans are supplied high Nitrogen and Potassium fertilizers for initiate flowering (High Potassium and Nitrogen) and maintain of flowers. Several trade names are available for above three growth stages of plants. In commercial nurseries additional micro nutrition's Organic fertilizers and weed extracts are supplied according to growers experience and knowledge (Albert Solution, Bloom Special, Maxicrop, Osmocote).

Some Ago-Chemicals Used in the Nursery


FARMERS MANCOZEB
mancozeb 80% (w/w) WP


PENTAGON CAPTA
captan 50% (w/w)

11. Training Institutes

a. Department of Crop Science
Faculty of Agriculture
University of Peradeniya.
Contact No : +94 81 239 5110 / 12
E-mail : agricropdept@gmail.com

b. Department of National Botanical Garden,
P O Box 14, Peradeniya
Contact No : 081 238 8088
E-mail : deptnbg@gmail.com

c. SLIIT Malabe Campus
New Kandy Road, Malabe
Contact No : 011 7544801
E-mail : info@sliit.lk

d. District Training Center (Western Province)
Pitipana North, Gabadawatta, Homagama.
Contact No : 0112 855 745

e. District Agriculture Training Centre (Western Province)
Alawwa Road, Ambepussa
Contact No : 0332 273 359

12. Cost Components for Low Cost Tissue Culture Techniques Household Level

Table 1 : Cost Components for Low Cost Tissue Culture Techniques

	Item	Expected cost Rs.
1	Room preparation	60,000
2	Racks installation	50,000
3	Air condition unit installation (12,000 BTU)	120,000
4	Supply of refrigerator	75,000
5	Pressure cooker	18,000
6	pH meter	15,000
7	Glassware	60,000
8	Chemicals	100,000
9	Digital balance	80,000
10	Glass box	7,000
11	**Total initial Capital**	**585,000**
12	**Maintenance cost borne by the Applicant**	**100,000**

Estimates are done for 10 x 10 sq ft lab unit, generally 1500 - 2000 bottles.

13. Income Projection

Table 2 : Income Projection with Production and Pricing

2024	Unit price Rs.	Quantity	Sale value Rs.
1) Anthurium: Lady Jene			
a)Community pots	1000	100	100,000
b)Small plant-lets	100	2500	250,000
c)Medium plants	175	2000	350,000
e) Flower bearing plants	250	1000	250,000
		5600	950,000.00
2)Orchids			
a)Community pots	1500	200	300,000
b)Small plant-lets	120	1200	144,000
c)Medium plants	200	1000	200,000
e) Flower bearing plants	500	500	225,000
		2900	869,000.00
Total (2022)		8500	1,819,000.00
2023 (10% increment)			2,000,900.00
2024 (20% increment of			2,182,800.00

Some Selected Images of Low Cost Tissue Culture Techniques for Anthurium

- Inside View of the Low Cost Lab (Racks and Glass Box)

Close View of Racks with Bottles

Different Stages of Growth

Anthurium Plantlets Growing on Trays and Cocopits (Different Nursery Stages)






Latter Stages of the Growth on the Pots (Ready to sell or Plant in the Pots)

View Inside the Plant House (Ready to sell or Plant in the Pots)

Pigtail Verities

Seedling Plants

- SUCCESS STORIES -

Application of Low Cost Tissue Culture Techniques for Floriculture

Successful Entrepreneur in Floriculture

Name : Ms. Niluka Sewwandi Fernando

Address : 5th Mile Post, Rassagala, Balangoda

Business Name : Nilu Flower Garden

Business Type : Floriculture - Anthurium Maintaining a own low cost tissue culture lab (House hold)

Contact No. : 0773146889

Name : Mr. U.D. Amila Lakmal

Address : Peella Walakada, Gagalaga, Baduraliya

Business Name : Evergreen Agriculture (pvt) Ltd

Business Type : Floriculture and Horticulture

Contact No. : 0771131656

Name : Ms. D. M. Priyanka Dinesha Dissanayake

Address : 35/A, Senasuma, Uyanwatta, Manikhinne

Business Type : Floriculture

Contact No. : 0776525810

REFERENCES

1). නාගහවත්ත, එස්.එම්; ශිරාණි, ඩී.ඒ.; කරුණානන්ද ඩී.පී. (2016), කෘෂි බෝග සඳහා පටක රෝපණය. කෘෂිකර්ම දෙපාර්තමේන්තුව. කෘෂි ප්‍රකාශන ඒකකය. ගන්නෝරුව.

2). Department of National Planning, (2016). Project Recommendation Letter.

3).https://www.srilankabusiness.com/floriculture/floriculture-export-erformance.html