PROPAGATION AND NURSERY MANAGEMENT OF FRUIT CROPS

HFS-503; 3(2+1)

PRACTICAL MANUAL

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Course: Propagation and Nursery Management in Fruit crops, HFS-503; 3(2+1)
Practical: Hands on practices on rooting of dormant and summer cuttings. Anatomical studies in rooting of cutting and graft union. Hands on practices on various methods of budding and grafting. Propagation by layering and stooling. Micro-propagation-explant preparation, media preparation, culturing-meristem tip culture, axillary bud culture, micro-grafting, hardening and visit to commercial tissue culture laboratories and accredited nurseries.

Name of Students: ……..

Roll No……..…… **Batch**……..

Session …….. **Semester**……..…….……....

Course Name ……..……..……

Course No: ……..…… **Credit:** ……..……..……..

Certificate

This is to certify that Shri./Km. ……..……...

ID No: ..... has completed the practical of course …….. courses No ..... as per the syllabus of M. Sc (Horticulture) Fruit Science .....semester in year …….. in the respective lab/field of college.

Date: **Course Teacher**

CONTENTS

Exercise No: 1

Objective: To identify basic tools and laboratory equipment used for plant tissue culture.

Since in vitro propagation is a very labor-intensive process and regeneration potential of explants depends on number of factors, the basic set up of laboratory and standardization of the specific protocols for different starting materials are important determinants of the final success of this process in a laboratory. The plant tissue culture techniques for most plant systems often require similar basic laboratory equipments. The following table enlists the items that are commonly required in a laboratory for in vitro propagation of plant materials:

S. No	Item/equipment's	Uses
1.	Water purification system:	
2.	Weighing balance:	
3.	pH meter:	
4.	Hot plate/stirrer:	
5.	Refrigerator and freezer:	
6.	Inverted microscope:	
7.	Liquid nitrogen (N2) freezer or cryostorage container	
8.	Centrifuge:	
9.	Water bath:	
10.	Incubator:	
11.	Cell culture hood:	
12.	Laminar flow transfer hood:	

13.	Glassware/Beakers:
14.	Wash bottles:
15.	Bottles:
16.	Brushes:
17.	Culture tubes:
18.	Culture tube racks:
19.	Closures:
20.	Culture vessel:
21.	Magenta B Caps:
22.	Culture vessels:
23.	Erlenmeyer flasks:
24.	Filtration system:
25.	Forceps:
26.	Graduated cylinders:
27.	Glass pipettes:
28.	Scalpel handles:

29.	Scalpel blades:
30.	Scoops:
31.	Spatula:
32.	Sterilizers, pressure cooker:
33.	Sterilizers, autoclave:
34.	Sterilizer:
35.	Stir bars:
36.	Stir bar retriever:
37.	Thermometers:
38.	Timer:
39.	Stocks of isopropyl alcohol:
40.	Detergent:
41.	Culture dishes:
42.	Chlorine bleach (Sodium hypochlorite):
43.	Roll tape:
44.	Gloves:

45.	Parafilm:
46.	Lab markers:
47.	Towels:
48.	Biohazard waste containers:

Assignment: List the various items of plant tissue culture laboratory and write its uses.

Exercise No: 2

Objective: To study the selection of mother plant for propagation

Materials and equipment: ……..……...

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Procedure: ……...…….

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Flow sheet for selection of mother plants for commercial propagation

1st Step-Orchard Map: ……....

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2nd step-Orchard Survey: ……...

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3rd Step-Preliminary inspection of the individual trees: ……..……..

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4th Step-selection of would be mother plants: ……..…….……...…
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5th Step-Selection of mother Plants: …….....
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6th Step-Selection of scion wood: ……..……
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Assignment: With the help of flow sheet select mother plants from the orchard.

Exercise No:3

Objective: To study the nursery bed preparation for rootstock seedling raising

Materials and equipment: ……

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Procedure: ……

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Preparation of beds:……

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Sterilization of nursery beds: ……

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Physical Methods: ……

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Chemical Methods: ……
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Sowing of seeds: ……
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Seed treatments: ……
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Irrigation: ……
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Care of seedling: ……
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Assignment:

Exercise No: 4

Title: To study the seed treatment, seed sowing and germination

A. Hot water treatments:

Materials and equipment: ……

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Procedure: ……

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B. Acid treatment

Materials and equipment: ……

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Procedure: ……

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C. Treatments with growth regulators and chemicals:

Materials and equipment: ……

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Procedure: ……

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Table1: Observations on germination of seeds.

Kind of seed	Pre-treatment	Date of sowing	date of observation	No of seeds				Germination (%)
				normal germination	Abnormal germination	Dead or decaying seeds	Polyembryonic seeds	
	Un-treated							
	Treated: Stratified							
	Scarified							
	Gibberellin							
	Fungicide							
	Heat treatment							

Exercise No: 5

Objective: To study about different type of cuttings for propagation

The process of propagation of plants by cuttings is known as cutting. A cutting is a part of a plant that will produce roots when put in soil media and eventually produces a new plant quite true to the parent plant.

Classification of cuttings: Stem cuttings, Root cuttings, Leaf cuttings

Materials required: ………………………………………………………………………………………...

Procedure: ………………………………………………………………………………………………
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Observations

S. No.	Types of cutting	No. of cutting planted/made	No. of cutting rooted	Percentage of rooted cutting	Average root length
1.	**Hard wood**				
2.	**Semi hard wood**				
3.	**Soft wood**				

Exercise No: 6

Objective: To study about propagation through budding

Budding is also a method of grafting wherein only one bud with a piece of bark and with or without wood is used as the scion material. It is also called as bud grafting. The plant that grows after union of the stock and bud is known as budding.

Materials required: ……...

Procedure: ……

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Draw labelled diagrams of each type of budding

Exercise No: 7

Objective: To study about propagation through grafting

Grafting is an art of joining parts of two independent plants in such a manner that they unite and grow together into single independent plant. The part of graft combination which is to become the upper portion or the shoot system or top of the new plant is termed the **scion** and the part which is to become the lower portion or the root system is the **rootstock** or **under stock** or some time **stock**. The single plant obtained as a result of union between the stock and scion is termed as **Stion**.

Materials required: ……...

Raising rootstock: ……..……..

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Collection of scion: ……..

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Procedure of grafting: ……..

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Observations

S. No. of plants	No. of graft sprouted	Graft success %	No. of leaves per graft	Scion girth	Fresh root biomass	Dried root biomass
1.						
2.						
3.						
4.						
5.						

Draw labelled diagrams of each type of grafting

Exercise No: 8

Objective: To study the anatomical observation of graft union

Material required: ……

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Procedure: ……

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Observations: ……………………………………………………………………………………..

Exercise No: 9

Objective: To study the shoot tip grafting *in vitro*

Citrus graft-transmissible diseases produced by viruses, viroids, some bacteria, spiroplasmas, and phytoplasmas result in serious economic losses in most citrus growing regions of the world. Pathogen-free plants of many cultivars are often not available and it is necessary to recover healthy plants from infected ones. In this situation, a method able to recover citrus plant free of all graft-transmissible pathogens and without juvenile characters was required to produce healthy trees for commercial propagation.

Murashige *et al*. (1972) was able to recover a few citrus plants by grafting shoot tips from diseased plants on young rootstock seedlings growing in vitro. Some of these plants were free of the exocortis viroid and did not have juvenile characters. This procedure was studied in detail by Navarro *et al.* (1975), who named it shoot tip grafting in vitro (STG), and developed a routine procedure that allowed a 30-50% incidence of successful grafts that were transplanted to soil, with over 95% survival rate. The resulting plants did not have juvenile characters, and most of them were free of graft-transmissible pathogens.

Technique of shoot tip grafting *in vitro*

Rootstock Preparation: ……..

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Scion Preparation: …….

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Grafting:

Culture *In Vitro* of Grafted Plants:

Transplanting to Soil:

Exercise No: 10

Objective: To study about propagation through layering

Layering is the developing of roots on a stem while it is still attached to the parent plant. The rooted stem is then detached or become a new plant growing on its own roots. A layered stem is known as a layer.

Mound layering:

Materials required: ……………………………………………………………………………………..

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Procedure of Mound (stool) Layering: …………………………………………………………………

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Air layering

Material required: ……………………………………………………………………………………….

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Procedure of Air layering: ………………………………………………………………………………..

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Observation

Crop name	Date of air layering	No. of air layers made	Date of separation	No. of Air layers rooted	Average no. of roots per layer	Average length of roots

Draw labelled diagrams of each type of layering

Exercise No: 11

Objective: To study the preparation in inoculation

Explant is any portion of plant taken for vitro culture. It may be a portion of the shoot, leaves, or some cells. Any part of the plant that is able to regenerate and give rise to a callus can be used as an explant. For in vitro culture the explant is first selected and then prepared for inoculation as per the standardized protocols.

Requirements

Reagents & Chemicals: Tween 20 (liquid detergent), 0.1% $HgCl_2$, 70% alcohol, sterile distilled water

Glassware's: All the glassware's should be sterile like Blades, Petri plates, Beakers, Forceps, Muslin cloth

Equipment: Autoclave and Laminar airflow hood

Selection of explant:

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Surface sterilization of explants:

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Commonly used chemicals sterilant:

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Explants after treatment with sterilants must be thoroughly rinsed with sterile distilled so that there is no adverse effect of toxic chemicals in establishment of culture.

Procedure

Washing of explant with tap water to remove soil and dust

Transferring washed explant into a glass beaker with drops of liquid detergent (Tween 20)

Covering beaker mouth with muslin cloth with the rubber band

Keeping under running tap water for at least one hour to remove any waxy deposition on explant surface

Washing with distilled water at least three four times

Transferring the explant into laminar airflow hood

Washing the explant with sterile distilled water (3-4 minutes) for three to four times

Treating explant with 0.1% $HgCl_2$ solution for one minute

Washing with distilled water at least three four times

Washing with 70% alcohol for one minute for removing water from the surface of explant

Transferring the sterile explant to a sterile petriplate

Cutting the leaf into small pieces of about 1x1 cm with sterile blade

Explant ready for inoculation

Exercise No: 12

Objective: Media preparation for *in vitro* culture

Media is the source through which the plants receive nutrients for good and healthy growth. It is a combination of different components which provide different nutrients at different stages of plant growth. The success of in vitro culture depends on the nutritional requirements of the cultured cells and tissue composition of the cultured media. The basic nutritional requirement consists of inorganic salts, carbon and energy source, vitamins and phytohormones and also organic nitrogen compound, organic acids. Media composition was therefore formulated considering specific requirements of particular system.

Requirements

Chemical: Stock solutions, distilled water, sucrose, myo-inositol, agar-agar, polyvinyl pyrrolidone

Glassware: Culture vessels, funnel, beaker, glass rod, filter paper, pipette, measuring cylinder

Instrument: Autoclave, pH meter

Composition of Murashige and Skoog (1962) basic salt solution (M&S)

NOTE:

- All stock solution should be clear and transparent, free of dust and without precipitation
- Stock solutions should be stored in amber colour bottles.
- Stock solutions are kept at 4˚C in dark room. These solutions are stored for limited period of times
- After using stock solution immediately keep close and don't expose for longer time

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Exercise No: 13

Objective: To study about meristem culture

The main objective of meristem –tip culture is the production of disease-free plants through micro propagation. Most of the horticultural crops are infected by systemic disease caused by fungi, viruses, bacteria, Mycoplasma and nematode. Plant infected with bacteria and fungi may be treated with bactericides and fungicides, there is no commercially available treatment to cure virus infected plants. It is possible to produce disease free plants through apical meristems tissue culture. Meristem –tip cultures has also enabled plants to be freed from other pathogens including Viroids, mycoplasmas, bacteria and fungi.

Reasons of meristems for virus invasion are:

- Viruses move readily in a plant body through the vascular system which in meristems is absent
- A high metabolite activity in the actively dividing meristematic cells does not allow virus replication
- A high endogenous auxin level in shoot apices may inhibit virus multiplication.

Procedure:

Cutting the tip portion of the twig from healthy plant

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Surface sterilize the shoot apices in a sodium hypochlorite solution for 10 minutes

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The explants are thoroughly rinsed 4 times in sterile distilled water

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Transfer each explant to a sterilize petridish

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Remove the outer leaves from each shoot apices with forceps and the apex is exposed

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Transfer less than 1 mm in length (apex) to the surface of the agar medium

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Flame the neck of culture tube before and after the transfer of excised tips

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Incubate the culture under 16 hrs light at 25 ^{0}C

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After development of shoots transfer them to hormone free medium to develop roots

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The plants are transferred to pots

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The plants are shifted for hardening in hardening chambers

Exercise No: 14

Objective: To study about axillary bud culture

In the axillary bud method, isolation of a shoot tip is achieved from which the axils of leaves develop axillary buds under the effect of high concentrations of cytokinin. Apical dominance is suppressed under the effect of high cytokinin concentration and permits the development of axillary buds. Often the ratio used in the axillary bud method between cytokinin and auxin is 10:1. If a number of side shoots or axillary shoots have been formed on the shoot tip then their inoculation may be done on the fresh medium containing cytokinin. Often the axillary method is used in combination with the single node method. Substituted pyridylphenylurea compounds and thidiazuron stimulate axillary branching in wide range of species.

Procedure: ……..

Exercise No. 15

Objective: To study the hardening of tissue culture plants

The ultimate success of tissue cultured plants on a commercial scale depends on the ability to transfer these plants from a controlled, aseptic environment to land successfully with high survival rate. Hardening is a method in which the tissue culture plants developed in artificial media are habituated to grow in natural environment. The process of acclimatization of the in vitro grown plants to the normal environment is called hardening. Acclimatization is the adaptation of organisms to a new environment. When tissue culture plants are transferred from the lab to soil they are exposed to abiotic stresses, like altered temperature, light intensity, and humidity conditions, and biotic stresses, like soil microflora (microbes living in soil). So, they need step-wise acclimatization to successfully establish themselves in the natural environment.

Types of hardening

Primary hardening: The tissue culture developed plants are taken out from nutrient media and washed thoroughly with water. Then these plants are grown in netted plastic pots filled with liquid nutrient medium and kept in green house for 6 – 8 weeks.

Secondary hardening: Afterwards the plants are transferred to polybags filled with potting mixture and grown under shaded house for 6 – 8 weeks. After secondary hardening the plants are suitable for growing in farmer's fields.

Describe the establishment and erection of hardening structure

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Exercise No. 16

Objective: To visit to commercial tissue culture laboratories

Tissue culture, a method of biological research in which fragments of tissue from an animal or plant are transferred to an artificial environment in which they can continue to survive and function. The cultured tissue may consist of a single cell, a population of cells, or a whole or part of an organ.

Report of the visit:

Exercise No. 17

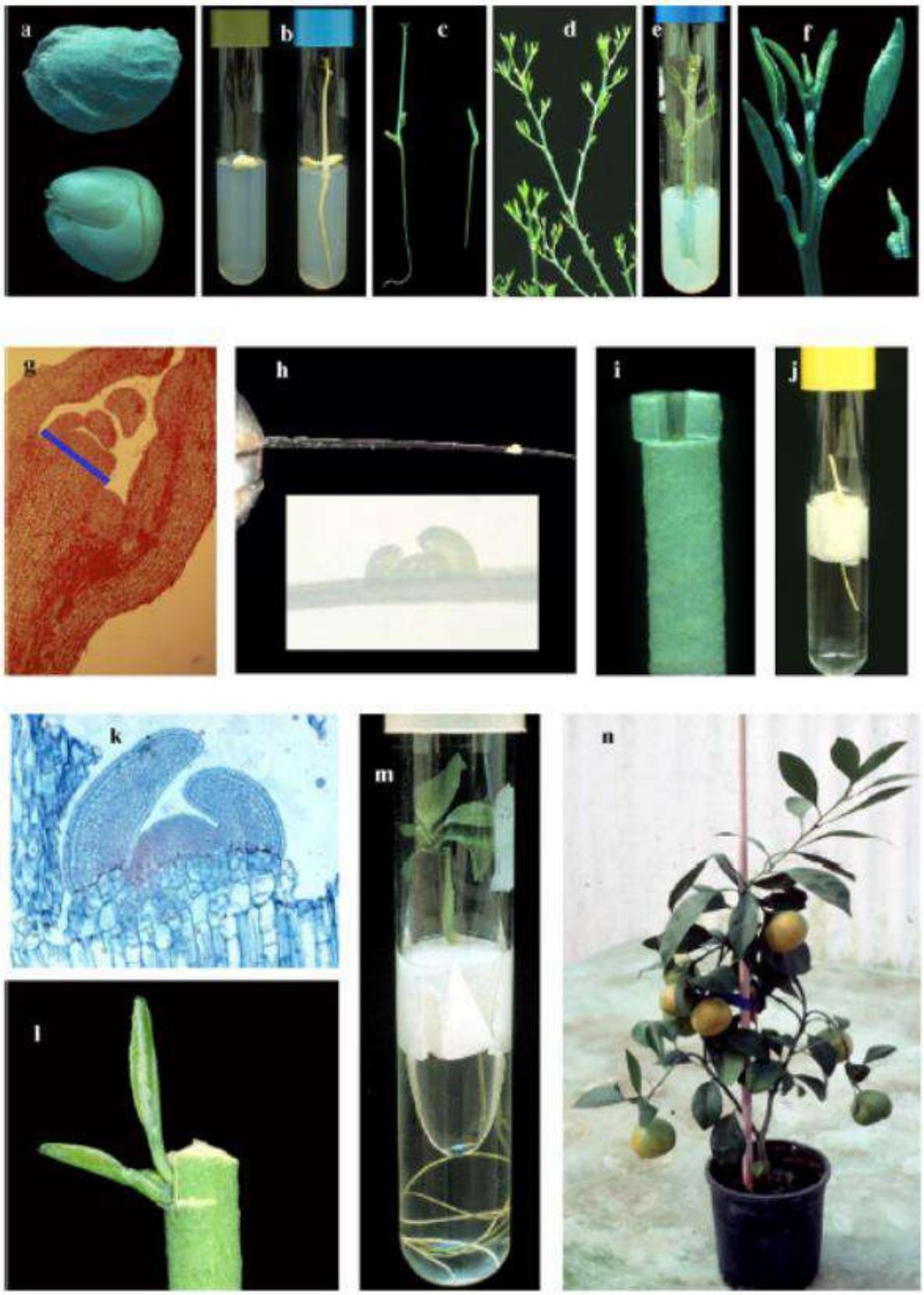
Objective: To visit to commercial horticulture Nursery

Commercial nurseries produce and distribute woody and herbaceous plants, including ornamental trees, shrubs, and bulb crops. While most nursery-grown plants are ornamental, the nursery business also includes fruit plants and certain perennial vegetables used in home gardens (e.g., asparagus, rhubarb).

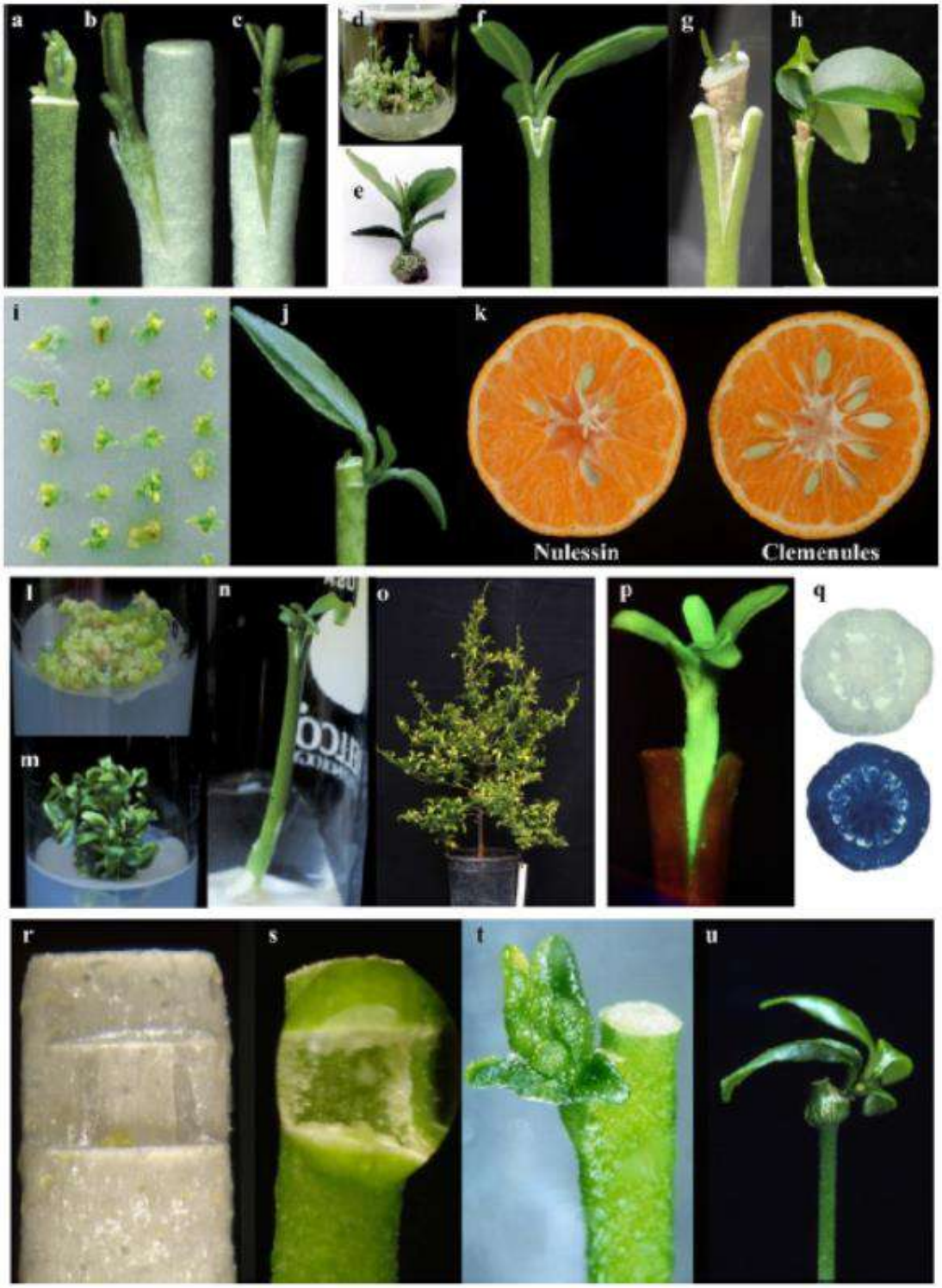
Report of the visit:

APPENDICES

CITRUS SHOOT-TIP GRAFTING IN VITRO



Standard shoot-tip grafting in vitro technique description. a-c. Rootstock preparation. d-f. Scion preparation. g-m. Grafting and culture in vitro of grafted plants. n. Plant recovered by STG.



a-c. Different types of STG in vitro for propagation of elite genotypes. d-f. Abnormal embryos produced after protoplast fusion experiments and grafted plant in vitro. g-h. STG from embryos with only root development and recovered from protoplast fusion. i-j. Regeneration of plants from irradiated shoot tips. k. Original 'Clemenules' clementine and selected irradiated 'Nulessin' clementine with reduced fertility. l-m. Abnormal proliferation of embryos from aborted seeds produced after in situ parthenogenesis. n-o. Regeneration of haploid 'Clemenules' clementine plant selected for whole citrus genome sequencing. p. Transgenic shoot grafted on an untransformed 'Troyer' citrange rootstock. q. 'Pineapple' sweet orange fruits from adult control (up) and transformed plants (down). r-u. Production of stable tetraploid plants of non-apomictic genotypes. r. Window incision in a rootstock on which a 'Clemenules' clementine shoot-tip was deposited. s. Drop of a colchicine solution applied seven days after micrografting. t-u. Successful grafts of tetraploid plants.

References

Murashige, T., Bitters, W.P., Rangan, T.S., Nauer, E.M., Roistacher, C.N. and Holliday, B.P. 1972. A technique of shoot apex grafting and its utilization towards recovering virus-free citrus clones. HortScience 7:118-119

Navarro, L., Roistacher, C.N. and Murashige, T. 1975. Improvement of shoot tip grafting in vitro for virus-free citrus. J. Am. Soc. Hort. Sci. 100:471-479