Draft Study Material

PLANT TISSUE CULTURE TECHNICIAN

(QUALIFICATION PACK: Ref. Id. AGR/Q8101)

SECTOR: AGRICULTURE

Grades 12

विद्यया ऽमृतमश्नुते

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NCERT

PSS CENTRAL INSTITUTE OF VOCATIONAL EDUCATION

(a constituent unit of NCERT, under MoE, Government of India)

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Preface

Vocational Education is a dynamic and evolving field, and ensuring that every student has access to quality learning materials is of paramount importance. The journey of the PSS Central Institute of Vocational Education (PSSCIVE) toward producing comprehensive and inclusive study material is rigorous and time-consuming, requiring thorough research, expert consultation, and publication by the National Council of Educational Research and Training (NCERT). However, the absence of finalized study material should not impede the educational progress of our students. In response to this necessity, we present the draft study material, a provisional yet comprehensive guide, designed to bridge the gap between teaching and learning, until the official version of the study material is made available by the NCERT. The draft study material provides a structured and accessible set of materials for teachers and students to utilize in the interim period. The content is aligned with the prescribed curriculum to ensure that students remain on track with their learning objectives. The contents of the modules are curated to provide continuity in education and maintain the momentum of teaching-learning in vocational education. It encompasses essential concepts and skills aligned with the curriculum and educational standards. We extend our gratitude to the academicians, vocational educators, subject matter experts, industry experts, academic consultants, and all other people who contributed their expertise and insights to the creation of the draft study material. Teachers are encouraged to use the draft modules of the study material as a guide and supplement their teaching with additional resources and activities that cater to their students' unique learning styles and needs. Collaboration and feedback are vital; therefore, we welcome suggestions for improvement, especially by the teachers, in improving upon the content of the study material.

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Date: 30 March 2025

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Module 1
Preparation of Mother Plant and Explant for Plant Tissue Culture

Module Overview

Preparation of Mother Plant and Explant for Plant Tissue Culture forms the foundational step in ensuring the success of any plant tissue culture process. The selection of a healthy mother plant and the careful preparation of explants are crucial to achieving contamination-free and responsive cultures.

This module provides a comprehensive overview of the initial procedures involved in plant tissue culture. Session 1 focuses on the selection and pre-treatment of the mother plant, highlighting the key factors that affect explant quality, such as plant health, physiological stage, and growing conditions. Session 2 introduces students to the fundamental tissue culture techniques used for initiating cultures from selected explants. This includes procedures like explant excision, surface sterilization, and inoculation under aseptic conditions.

Learning Outcomes

After completing this module, you will be able to:

- Explain the criteria for selecting a mother plant and describe the pre-treatment steps required for successful tissue culture.
- Identify and differentiate between various plant tissue culture techniques and understand their specific applications in plant propagation.

Module Structure

- Session 1: Selection and pre-treatment of Mother Plant
- Session 2: Different Plant Tissue Culture Techniques

Session 1: Selection and Pre-treatment of Mother Plant

The first basic step in plant propagation through tissue culture is the selection of a healthy plant, which is known as the mother plant. A mother plant provides the initial materials for regeneration in an artificial culture medium under controlled environmental conditions. The plant parts taken from the mother plant are known as explants and may be in the form of shoot tips, stems,

leaves, meristems, roots, etc. The explants taken from the mother plant should be viable and free from insects, pests and diseases. The aim of selecting a healthy mother plant is to develop genetically identical plantlets (true-to-type) and exhibit desirable traits similar to those of the mother plant. The selection of superior mother plants is essential for large-scale *in vitro* propagation, especially in the context of commercial production.

Criteria for the selection of the mother plant

- The selected mother plants should be healthy, true-to-type, free from physical damages, diseases and pests, especially virus diseases.
- The selection criteria should focus on a few important phenotypic traits that are estimated to have a relatively high heritability, such as stem straightness, branching and flowering habits.
- The mother plant should possess the desired characteristics, such as high yield, good quality, and specific traits relevant to the purpose of propagation.
- The grower should have detailed information about the mother plant. A Pedigree record and source of each mother plant should be maintained and catalogued.
- The tissues selected from mother plants should be collected in an ice box and transported to tissue culture production facilities on the same date of collection.
- Mother plants should be grown under roofless, insect-proof shade nets with adequate height.
- Mother nursery must be located away from other plantations of the same species, maintaining an appropriate isolation distance to maintain genetic purity and prevent the spread of virus diseases.
- Mother plants should be grown under optimal management conditions to ensure the true expression of desirable traits. Each plant should be tagged with a master code number so that the plantlets developed could be traced back to the mother plant.

Establishment of the mother nursery

Establishment of a mother nursery for tissue culture is a fundamental step in *in vitro* plant propagation. The selected mother plants, which are elite clones with proven yield potential and improved agronomic or horticultural traits, should be thoroughly screened before planting in a mother nursery. They should be planted in an isolated area /or under protected conditions, such as a glasshouse.

The healthy plants shifted in protected conditions should be properly labelled with an accession number, plant species/variety and date of planting. The details of the particulars should be recorded in a master stock register maintained by the facility. Planting in the mother nursery, each plant should undergo testing to ensure that it is virus-free and maintained in a virus-free condition until it is used for tissue culture production. The establishment of a mother plant nursery strengthens the commercialization of tissue culture for the large-scale production of plants under controlled conditions.

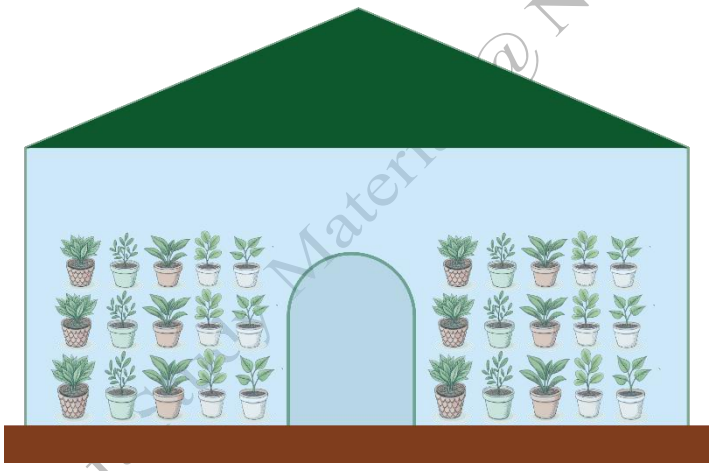

Fig. 1.1: Establishment of a mother plant nursery in protected conditions

Pre-treatment of the mother plant

- **Pre-treatment with Fungicides and Insecticides**: To ensure the success of the tissue culture process, it is essential to apply fungicides and insecticides to mother plants a few days before explant collection. This pre-treatment allows the chemicals to effectively control fungal infections and insect pests while minimising the risk of harmful chemical residues on the explants. Systemic fungicides and insecticides are often preferred as they are absorbed by the plant, providing prolonged protection against pathogens and pests.

- **Pruning:** It is an important step in preparing mother plants for tissue culture. By removing older leaves, flowers, or damaged tissues, pruning stimulates the new growth, which is more suitable for explant collection. Younger tissues, such as the apical meristem or young stems, have better regenerative potential and respond more effectively to growth regulators used in tissue culture. It improves the quality of explants. Removing non-essential or damaged parts by pruning allows the plant to redirect its resources for promoting stronger growth and higher-quality explants.

- **Nutrient Optimisation:** Ensuring that the mother plants are well-nourished before explant preparation is important. Adequate watering and fertilization maintain plant vigour and improve the quality of explants for tissue culture.

Prerequisite for micropropagation

- Micropropagation involves the establishment of aseptic culture, which is achieved by disinfection, excision and incubation of explants.

- Sodium Hypochlorite (NaOCl) is the common disinfectant, and the laboratory grade is normally used at concentrations ranging from 0.5-1.0%.

- Sucrose is the most preferred carbon source used at a concentration of 2-4% (W/V). Vitamins, thiamine, Nicotinic acid and pyridoxine are frequently used.

- Amino acid glycine is being used as an immediate source of nitrogen for cultured tissues. Commonly used reducing agents are ascorbic acid and citric acid (1.0 and 1.5% W/V, respectively), which are used in various stages, like disinfecting solution itself or after disinfection.

- Auxins and cytokinins are commonly used for rooting and shooting, respectively.

- The widely used auxins are Indole-3-Acetic Acid (IAA), Naphthalene Acetic Acid (NAA) and ndole-3-Butyric Acid (IBA). Benzylaminopurine (BAP) is the cytokinin of choice for *In vitro* shoot bud proliferation. pH is usually maintained at 5.8.

- The optimum incubation temperature should be between 24-26°C.

- Generally, the light intensity is maintained in range of 1,500-3,000 lux. Higher levels of 3,000-10,000 lux during the later stages improve the survival rate of plantlets upon transfer to soil.

Preparation of Explants

- The explants taken for tissue culture should be healthy and disease-free. The cutting tools used for excising plants should be disinfected with 70% alcohol, and the hands should be properly washed with a suitable detergent.

- The working surface of the laminar air flow cabinet should be disinfected with 70% alcohol or sterile filter paper pads.

- The explants should be sterilized by immersing in an appropriate sterilant solution, such as 0.1% - 0.2% Sodium hypochlorite or mercuric chloride*($HgCl_2$) solution for 5 to 20 minutes. They are then washed 3 to 4 times in sterile distilled water before being inoculated onto the culture media, depending on the types of tissue. For meristem tissue culture plants, the explants of 1.0 to 1.5 mm are ensured from the shoot-tip using a sterile scalpel and then transferred onto the culture media using sterile forceps (tweezers).

- The culture bottles are appropriately labelled, giving information about accession number, plant species/variety, date of transfer and name of technician.

- The culture bottles are then incubated in growth rooms under controlled temperature/humidity/conditions for the proliferation of shoot buds.

- The proliferated shoot buds in clumps are individually transferred to another nutrient medium for shoot elongation and root development.

- The stock cultures thus obtained are maintained in a refrigerator, and the particulars of stock cultures maintained will be recorded in a stock culture register.

- The stock cultures will be utilised for further multiplication to obtain the desired number of plantlets.

Source: *Standard Operating Procedures For Recognised Tissue Culture Production Facility, National Certification System for Tissue Culture Raised Plants (NCS-TCP), Department of Biotechnology, Government of India, New Delhi*

**Use of mercuric chloride should be avoided. Several research labs around the world have completely banned the use of mercuric chloride. In India as well its use is also banned except for seed treatment of potato and sugarcane..*

Practical Exercises

Activity 1

Visit tissue culture mother block and find out the Criteria for Selecting Mother Plants

Materials required: Pen, pencil, notebook, magnifying lens, etc.

Procedure:

1. Visit a tissue culture mother block and discuss with the expert/teacher and note down following:
   - Selection of desired crop plant
   - Name of the cultivar/variety
   - Age of the plant
   - Crop Season
   - Explant to be taken
   - Health of the plant
   - Precautions taken during selection of the explant.
2. If any query, discuss with the expert and note it down.

Check Your Progress

A. Multiple Choice Questions

1. What is the primary aim of selecting a healthy mother plant?
   a) To increase diversity
   b) To develop genetically identical plantlets
   c) To produce hybrid plants
   d) To prevent flowering
2. Which substance is commonly used as a source of nitrogen in cultured tissues?
   a) Thiamine
   b) Ascorbic acid

c) Glycine
d) Citric acid

3. Which hormone is commonly used for rooting in tissue culture?

a) BAP
b) IAA
c) Thiamine
d) Nicotinic acid

4. In tissue culture, the preferred light intensity during the later stages is:

a) 500-1,000 lux
b) 1,500-3,000 lux
c) 10,000-15,000 lux
d) 3,000-10,000 lux

5. Explants are sterilized using mercuric chloride solution with a concentration of:

a) 0.1%-0.2%
b) 1%-2%
c) 2%-4%
d) 4%-6%

B. Fill in the Blank

1. The plant identified for tissue culture and used for *in vitro* propagation is called a _____.
2. The _____ is the part of the plant taken from the mother plant for tissue culture.
3. _____ is commonly used as a disinfectant in the pretreatment of explants.
4. The most preferred carbon source used in the culture medium is _____.

C. Subjective Questions

1. Define a mother plant and explain its role in plant tissue culture.
2. What are the key criteria for selecting a mother plant for tissue culture?
3. Describe the process of pretreatment and sterilization of explants.

Session 2: Different Plant Tissue Culture Techniques

Plant tissue culture techniques for clonal propagation

Naturally, the plants can be propagated by two methods: sexual reproduction through seeds or asexual reproduction using vegetative parts. Sexually propagated plants are highly heterogeneous in nature because their offspring are not genetically identical. In contrast, asexual reproduction gives rise to plants that are genetically identical to the mother plant. Multiplication of genetically identical individuals through asexual reproduction is called clonal propagation. Some crops, such as banana, grape, fig and chrysanthemum, produce little or no viable seeds; thus, vegetative multiplication is the only way to propagate such plants.

Clonal propagation of plants through *in vitro* techniques is called micropropagation. It is one of the robust techniques of plant tissue culture that enables the production of healthy and genetically identical plants using explants from the mother plants. This technique is generally used in agriculture, horticulture, forestry, and other plant-related research activities for large-scale production of plants with desirable traits, such as improved phenotypic characters, disease resistance and yield potential.

Such a technique of plant tissue culture offers a rapid and efficient production of plants under *in vitro* conditions using an artificial culture medium.

Steps of *in vitro* Clonal propagation (Micropropagation)

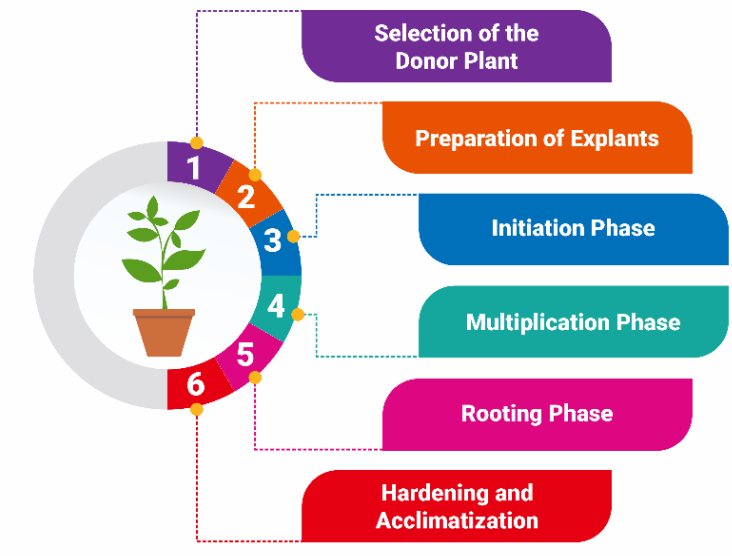

Fig. 1.2 –Steps of micropropagation

In vitro plant multiplication can be achieved via organised explant tissue or unorganised tissue originating from the explant. Organised explant tissue approaches include axillary bud proliferation (pre-existing meristems) and adventitious regeneration (culturing tissue without a pre-formed meristem). In contrast, plant regeneration from callus or cell cultures occurs without organised tissue, as detailed in the section below:

1- Multiplication by Axillary buds and shoot tips

Plants grown with their apical part as meristems are usually found in the tips of shoots and the axils of leaves. The meristem can produce new shoots, allowing the plant to develop new shoots. In natural conditions, branching is controlled by apical dominance, a process where the main shoot inhibits the growth of side shoots. This control is regulated by plant hormones. In tissue culture, scientists can modify these hormones in the growth media to promote the development of multiple shoots, bypassing apical dominance.

When shoot tips are cultured on a basic medium without added growth regulators, they grow as a single main shoot due to strong apical dominance. However, when hormones like cytokinins such as 6-Benzylaminopurine (BAP), N^6-(2-Isopentenyl) adenine (2iP) and zeatin are added to the medium, they promote the growth of axillary shoots, which form multiple shoots. These multiple shoots can then be divided into smaller groups or individual, shoots which can be cultured again to generate more clusters.

This technique is highly effective for clonal propagation, thus allowing the production of large numbers of genetically identical plants. With proper culture conditions and regular sub-culturing every 4-8 weeks, millions of plants can be generated from a single shoot within a year. It is particularly useful for plants that respond well to cytokinin treatments, ensuring healthy, undistorted growth without yellowing.

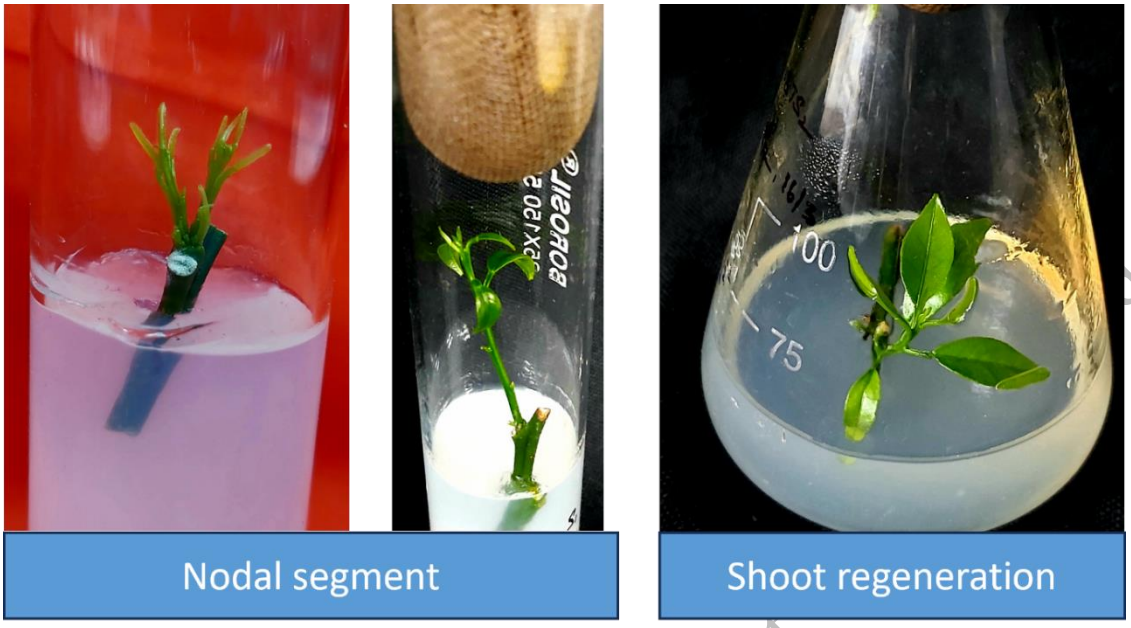

Fig. 1.3: Nodal segment

2- Multiplication by Adventitious Shoots

Adventitious shoots are shoots that grow from unusual parts of the plant, such as stems, leaves, or roots, instead of normal leaf axils and apical buds. Unlike axillary buds, which are naturally present as preformed meristems, adventitious shoots develop from tissues where meristems do not exist.

Adventitious shoots are grown from explants placed in the appropriate medium. Adventitious embryos, which are clonal structures similar to shoots, can also form plant tissues naturally or under controlled conditions. These techniques are used for clonal propagation in species like Citrus and Mango, where embryos develop from nucellar tissues. Orchids are also commercially propagated using these methods.

3- Multiplication through Callus Culture

Differentiating plants from cultured cells through shoot-root formation is one of the fastest methods for multiplying and cloning plants. However, plant tissues form a callus (an undifferentiated mass of cells), which is not ideal for large-scale propagation due to problems like genetic instability, which can reduce regeneration ability over time. Plant regeneration through callus cultures often involves specific cell layers. The outer meristematic layer contains genetically stable diploid cells that can develop into shoots or embryos maintaining totipotency. In contrast, the inner layers may

contain mixoploid cells, which are less stable. Calli can be cut or processed into thousands of small pieces, each capable of forming new shoots, making this process suitable for mass propagation.

4- Plant tissue culture techniques for somatic embryogenesis

Somatic embryogenesis is a plant tissue culture technique in which somatic cells (cells other than germ or reproductive cells) are used. Somatic embryogenesis is the process by which somatic cells or tissue develop into a differentiated embryo, and each fully developed embryo is capable of developing into a plantlet (a young or miniature plant). Embryos can be obtained directly from cultured explants, anthers or isolated single cells in culture. Somatic embryogenesis is a simultaneous but stepwise process of embryo development, progressing through various stages such as proembryo, globular, triangular, heart and torpedo stages.

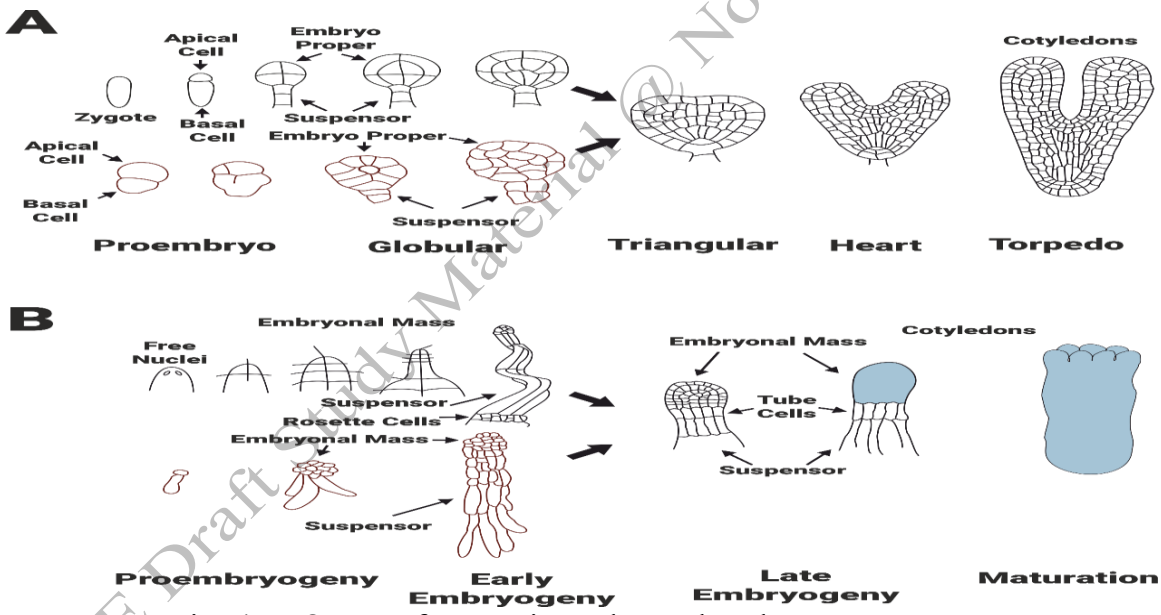

Fig. 1.4: Steps of somatic embryo development

Types of somatic embryogenesis

A- Somatic Embryogenesis in Dicotyledonous Cultures

1- Explant for Initiation of Embryogenic Callus:

Totipotent cells are special cells capable of developing into a whole plant. These cells are usually taken from small pieces of young plant tissues such as embryos or seedlings. For example, small parts of young flowers (before they are fully developed) can also be used to grow these cells in lab cultures. Other

plant parts, such as roots, leaves, and stems, can also be used. Within about three weeks, many embryos begin to form from the cells in the callus (a mass of undifferentiated plant cells). Mass of these special cells can also be grown from fully developed plant cells in a specialised culture medium. Somatic embryos can either grow directly or after being separated on a fresh nutrient-rich medium. An interesting feature is the formation of new embryos, called adventive embryos, on the surface of these embryos or on the plantlets grown from these somatic embryos. These adventive embryos, usually numbering between 5 to 50, develop from single surface cells.

Basic Requirements: To grow somatic embryos in a lab, certain important conditions need to be met, and this is usually done using suspension cultures. For the first time, somatic embryos were successfully grown in a lab in 1958, using carrot (*Daucus carota*) suspension cultures, by a scientist named Steward and his team.

I. **Auxin Supply**: Auxin is the major source of plant growth regulator incorporated in the culture medium for regeneration. It is very important for starting the process of somatic embryogenesis. Without auxin, tissues or callus cannot form embryos. The process of somatic embryogenesis occurs in two steps, i.e. callus formation and embryo development. The callus is grown and multiplied on a medium that has a high level of auxin (e.g., 2,4-D at 0.5 mg/L). This process helps to form small groups of special cells called Embryogenic Clumps (ECs), which can later develop into embryos. The ECs are then transferred to a medium with very little or no auxin (0.01–0.1 mg/L). This new medium, called the Embryo Development Medium (EDM), helps the ECs grow into mature embryos. The first medium with auxin is called the Proliferation Medium (PM) and helps in starting the process. Although other auxins such as 2,4-D are also used for this purpose.

II. **Nitrogen Source**: Nitrogen is very important for both starting and maturing somatic embryos. Plants require nitrogen in a reduced form, such as ammonium salts, for this process. A mix of reduced nitrogen (e.g., NH_4Cl) and nitrate (NO_3) works well in many cases. The source of Nitrogen can be coconut milk or casein hydrolysate, L-glutamine, L-alanine, NH_4Cl or NH_4NO_3. The commonly used MS medium (Murashige and Skoog) provides high amounts of nitrogen in the form of ammonium nitrate, which supports plant growth effectively.

III. **Establishment of Embryogenic Suspension Cultures**: To start a suspension culture (*Fig 1.6*), small pieces of callus tissue are placed in 50 ml of growth medium inside Erlenmeyer flasks. These flasks are gently shaken on a shaker platform at 125-160 rpm to ensure proper mixing and aeration. Another method uses a device called an auxophyton, which provides gentler agitation and aeration. Some other tools are also used for this process, like Tumble tubes (These tubes hold 10 ml of medium and are slowly rotated at 1 rpm), Nipple culture flasks (These larger flasks hold 250 ml of medium and rotate at the same speed). Both vessels work by lifting the tissue for aeration and then dipping it back into the medium, ensuring that the cells receive oxygen and nutrients. A typical procedure involves first growing the callus tissue in tumble tubes. Once the cells increase in number, they are transferred to larger flasks. For large-scale somatic embryogenesis, bioreactors—originally designed for microbial fermentation—are tested to grow tissues efficiently.

Embryogenic cell suspension develops within 2-3 weeks if the cells are regularly transferred to fresh medium. This helps prevent the cells from ageing too quickly and maintains their ability to form embryos. To subculture the cells, those in the active growth phase are allowed to settle for a few minutes, making it easier to remove most of the old medium. Then, about one-fourth to one-sixth of the cells are transferred to a new flask with fresh medium and shaken. If a finer suspension is needed, the cells can be sieved during transfer.

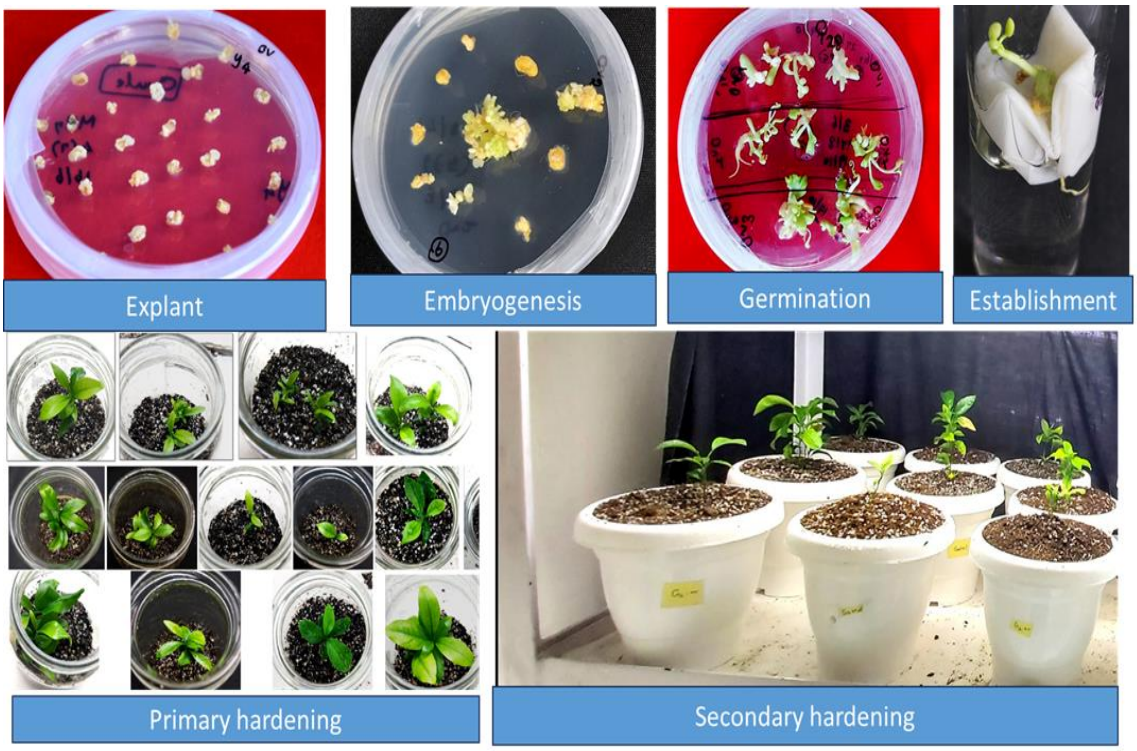

Fig. 1.5: Direct Somatic embryogenesis in dicotyledonous explant

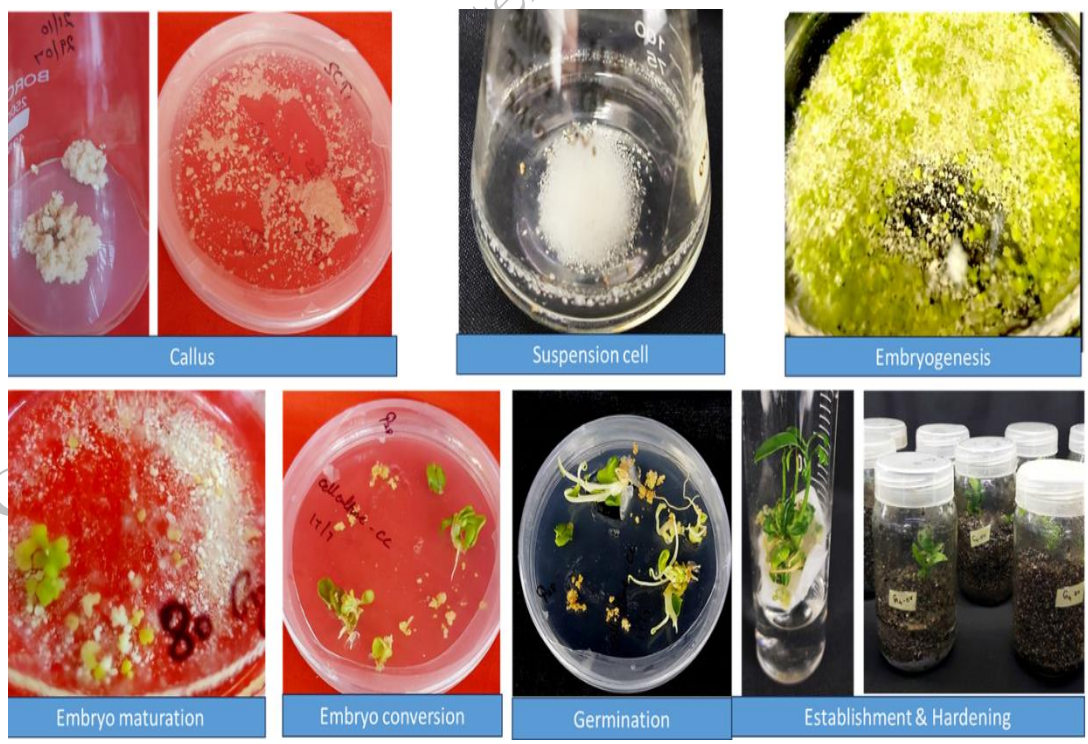

Fig. 1.6: Suspension cell culture-based somatic embryogenesis

B-Somatic Embryogenesis in Monocotyledonous Cultures

Many monocot plants, which are important in agriculture and medicine, do not easily regenerate new vegetative parts in lab cultures as dicot plants do. For this reason, it is best to take explants (small tissue samples) from specialised tissues, such as embryogenic or meristematic tissues, which are found in young flowers (inflorescences) or leaves. These tissues are better for producing new plants in cultures.

Selection of Explant

1- **Zygotic Embryo:** Young caryopses (10-15 days after pollination) are first sterilized by rinsing them in 70% ethanol for 30 seconds. Then, they are soaked for 10-20 minutes in 20% commercial bleach with a few drops of detergent added to help the solution spread. After sterilization the seeds are washed at least three times with distilled water, and the zygotic embryos (the embryos inside the seed) are carefully removed under sterile conditions. These embryos are placed in a culture tube with MS medium. After 4-6 weeks, a small, slow-growing callus (a mass of plant cells) will appear.

2- **Young Inflorescence**: The inflorescences, which are usually 1-2 cm long, are first sterilized. After sterilization, a vertical incision is made through the surrounding leaves to expose the inflorescence. The inflorescence is then cut into small 1-2 mm-thick segments. Each segment is placed on a growth medium containing 2,4-D, which helps promote the growth of an embryogenic callus (a mass of cells that can develop into embryos).

3- **Young Leaf:** The unexpanded leaves of young seedlings, which are grown from seeds in sterile conditions, are carefully removed. These leaves are then cut into small 1-2 mm-thick sections starting from the shoot meristem (the growth tip) and extending to the leaf apex (the top of the leaf). Six to eight of these small sections or explants are placed on a nutrient-rich medium to help them form a callus (a mass of cells that can develop into new plant tissue).

Induction of Embryogenic Cell Suspension: The obtained callus from the cultured explants is teased and broken into small pieces and placed in liquid medium. Once a strong embryogenic suspension (a culture with many cells capable of forming embryos) is established, somatic embryos can be formed. This can happen either by letting the culture age naturally or by transferring the

embryogenic tissue to a medium without 2,4-D. To encourage proper embryo development and prevent excessive root formation, the medium is typically supplemented with 500 mg/L glutamine and 0.1 µM zeatin or 0.1 µM ABA.

Orchard grass Embryogenesis: All monocot species can regenerate plants through somatic embryogenesis, and 2,4-D is the most effective hormone for producing embryogenic cultures. For successful induction and growth of somatic embryos, adding NH_4 (2.5-3 mm) and Casein Hydrolysate (CH) (3 g/L) to the medium is essential. While dicot somatic embryos are typically bipolar (having two distinct poles), monocot somatic embryos, such as those from grasses, begin as a complete ring of tissue but often develop asymmetrically. As they mature, these embryos form their first visible leaf. In contrast, a typical monocot zygotic embryo in monocots has a single fan-shaped cotyledon with a flattened top and round base. The mature monocot somatic embryo can grow on a semi-solid MS medium with added glutamine and zeatin.

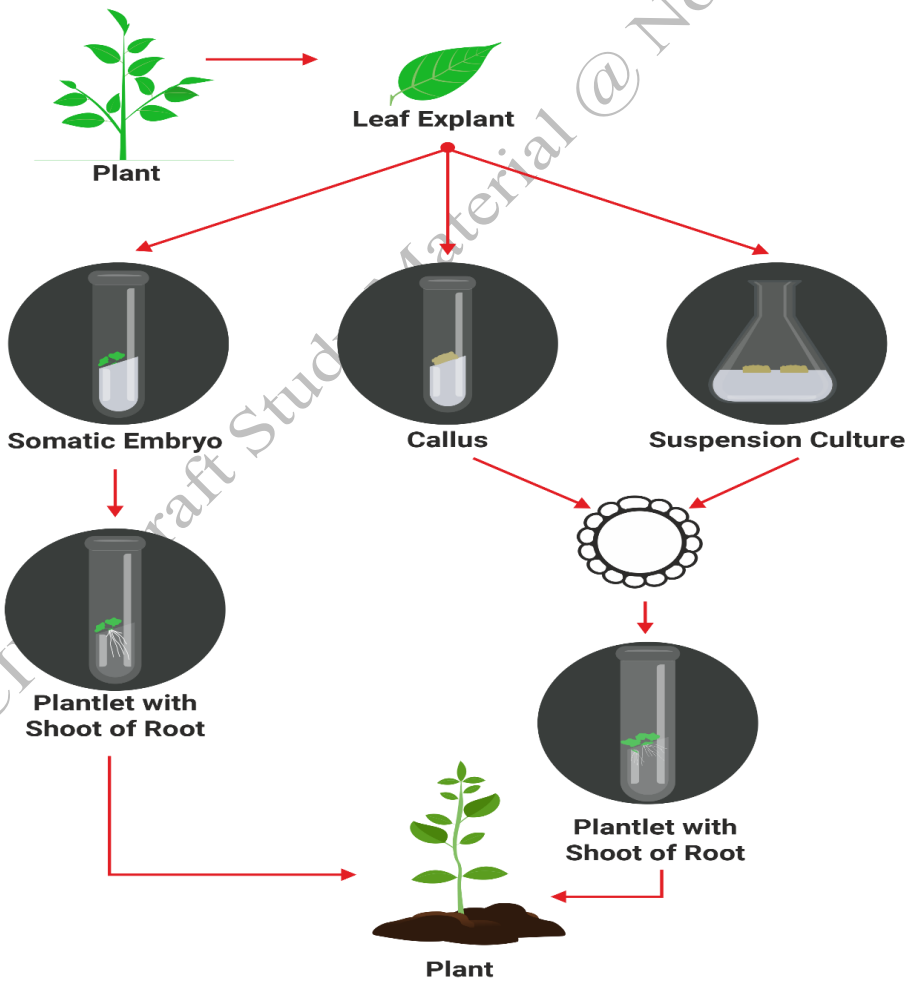

Fig. 1.7: Somatic Embryogenesis in plants

Plant Tissue Culture through Organogenesis

Organogenesis is the process of forming new plant organs, such as roots or shoots. This can be grouped into two categories, i.e., direct and indirect organogenesis. Indirect organogenesis is the process where new organs grow from a mass of plant cells called a callus, and direct organogenesis is where roots or shoots grow directly from a plant piece (explant) without forming a callus. The Indirect organogenesis process involves three steps: dedifferentiation, induction and differentiation. The dedifferentiation phase is when plant cells lose their specialised functions and become flexible, simple cells. In the induction phase, signals guide the cells to determine whether they will become roots, shoots, or other parts.

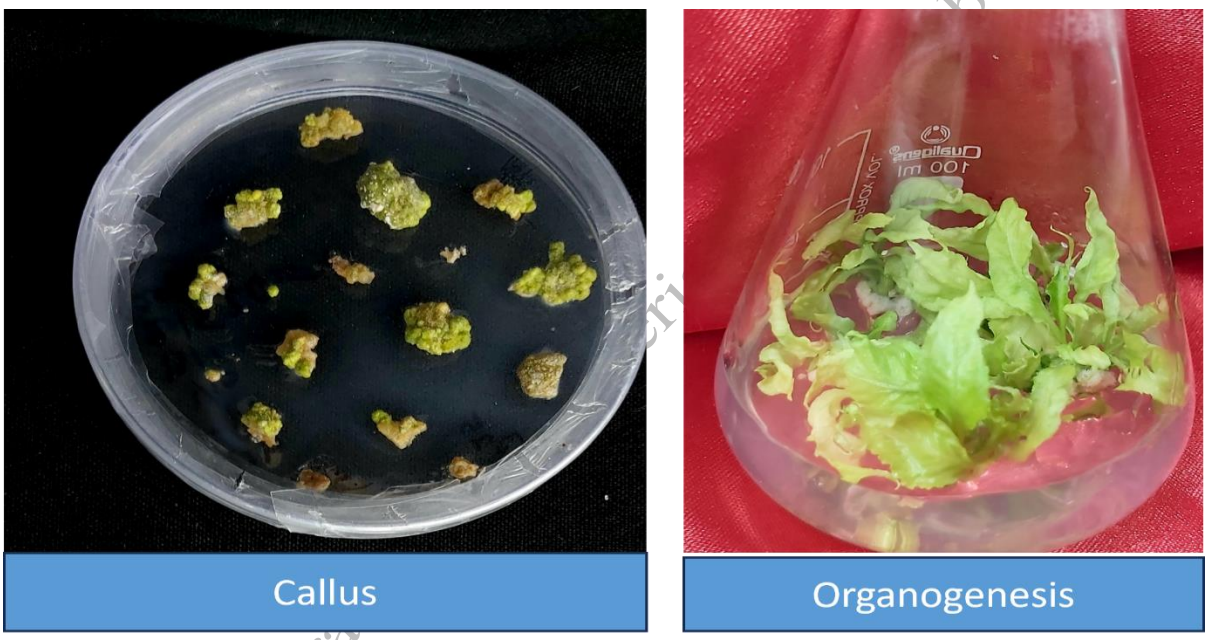

Fig. 1.8: Callus mediated organogenesis

Plant hormones also play an important role in organogenesis. A higher ratio of auxin to cytokinin promotes root formation, whereas a higher cytokinin to auxin ratio encourages shoot formation. Balanced hormone levels are essential for proper callus formation. The main difference between organogenesis and somatic embryogenesis depends on their structures and the hormones used. In organogenesis, the new organ connects to the existing vascular tissues, while somatic embryogenesis forms an independent structure called an embryo, which has both root (radicle) and shoot (plumule) but does not connect to the original plant tissues. Additionally, somatic embryogenesis requires only a single hormonal signal to produce a complete plant. Organogenesis, therefore, is a

versatile method that relies on manipulating plant cells and hormones to regenerate new parts.

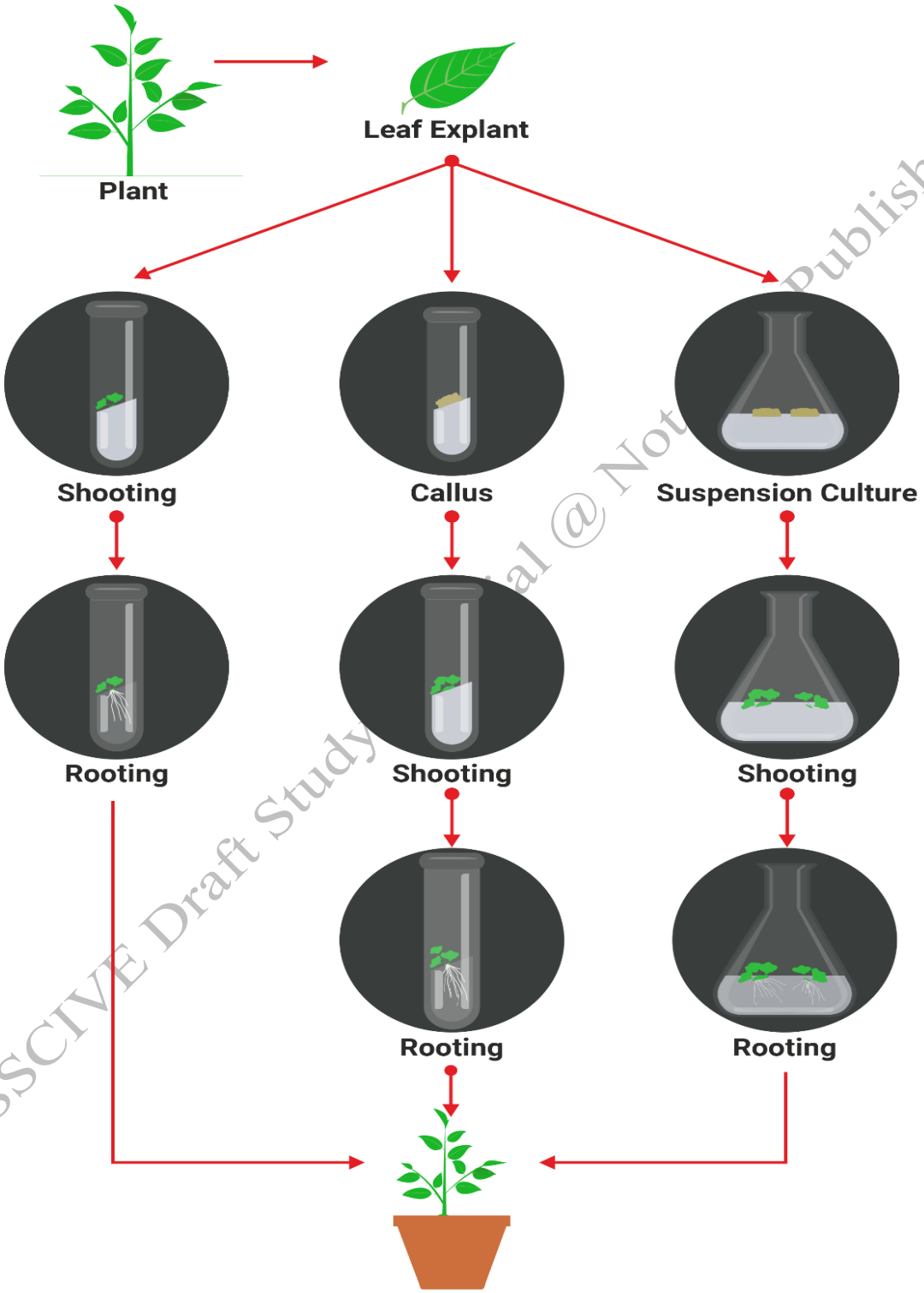

Fig. 1.9: Direct and Indirect Organogenesis in plants

Practical Exercises

Activity 1

Visit a Plant Tissue Culture Lab and familiarize yourself with the techniques involve in tissue culture.

Materials required: Pen, pencil, notebook, etc.

Procedure

1. Visit a nearby plant tissue culture laboratory.
2. Observe and note down the different tissue culture techniques being practiced, such as:
   - Micropropagation
   - Callus Culture
   - Embryo Culture
   - Protoplast Culture
3. Discuss with the expert to understand the purpose and steps of each technique.
4. Prepare a detailed note based on observations.

Check Your Progress

A. Multiple Choice Questions

1. Which of the following plants is commonly propagated through clonal propagation?
   a) Wheat
   b) Banana
   c) Corn
   d) Rice
2. Which hormone suppresses the growth of side shoots in plants?
   a) Cytokinin
   b) Ethylene
   c) Gibberellin
   d) Auxin
3. What is the first step in somatic embryogenesis?
   a) Callus formation

b) Organogenesis
c) Root development
d) Shoot elongation

4. What type of explant is ideal for initiating embryogenic callus in monocots?
   a) Mature leaf
   b) Embryogenic tissue
   c) Mature stem
   d) Woody bark
5. Which of the following techniques is ideal for plants that produce little or no viable seeds?
   a) Organogenesis
   b) Crossbreeding
   c) Clonal propagation
   d) Seed germination

B. Fill in the Blank

1. The process of producing multiple genetically identical plants using tissue culture is called _____.
2. _____ is the hormone commonly used to promote shoot formation in tissue culture.
3. The process of _____ involves forming a new plant organ, such as roots or shoots, from plant tissue.
4. Direct organogenesis forms roots or shoots without the formation of _____.

C. Subjective Questions

1. Describe Steps of Clonal Micropropagation.
2. Explain Organogenesis and its process.

Module 2
Inoculating the explant and its Multiplication

Module Overview

Inoculating the Explant and Its Multiplication is a vital stage in the plant tissue culture process, where the selected plant part (explant) is introduced into a sterile culture environment to initiate growth and multiplication.

This module offers a step-by-step understanding of how to handle and transfer explants for successful culture initiation. Session 1 focuses on the excision of explants from the mother plant, emphasizing techniques to minimize contamination and tissue damage during the cutting process. Session 2 guides students through the preparation and inoculation of explants into the culture medium, ensuring proper handling under aseptic conditions. By completing this module, students will develop the technical skills required for precise explant transfer and multiplication, laying the groundwork for further stages in plant tissue culture.

Learning Outcomes

After completing this module, you will be able to:

- Describe the process of excising explants from a mother plant, including sterilization and handling techniques.
- Demonstrate the preparation and inoculation of explants into a culture medium under aseptic conditions, ensuring successful culture initiation.

Module Structure

- Session 1: Excising of explant from Mother Plant
- Session 2: preparing and inoculating an explant to the culture medium

Session 1: Excising of explant from Mother Plant

An explant is the organ or small segment of the plant removed carefully from the mother plant to initiate *in vitro* culture under aseptic conditions in an artificial culture medium. Depending on the purpose of propagation, explants can be taken from various parts of the mother plants, such as the node, roots, stem, leaf, meristem, seed, flower or embryo.

Excision of the explant from the mother plant is a very important step and should be done carefully to avoid damage to the plant tissues. During excision, preventing contamination and preserving cellular viability are essential for the

proper establishment of callus and organogenesis from the explant. The explant excised from the mother plant is used for organogenesis. The steps involved in organogenesis and embryogenesis are explained in the figure given below:

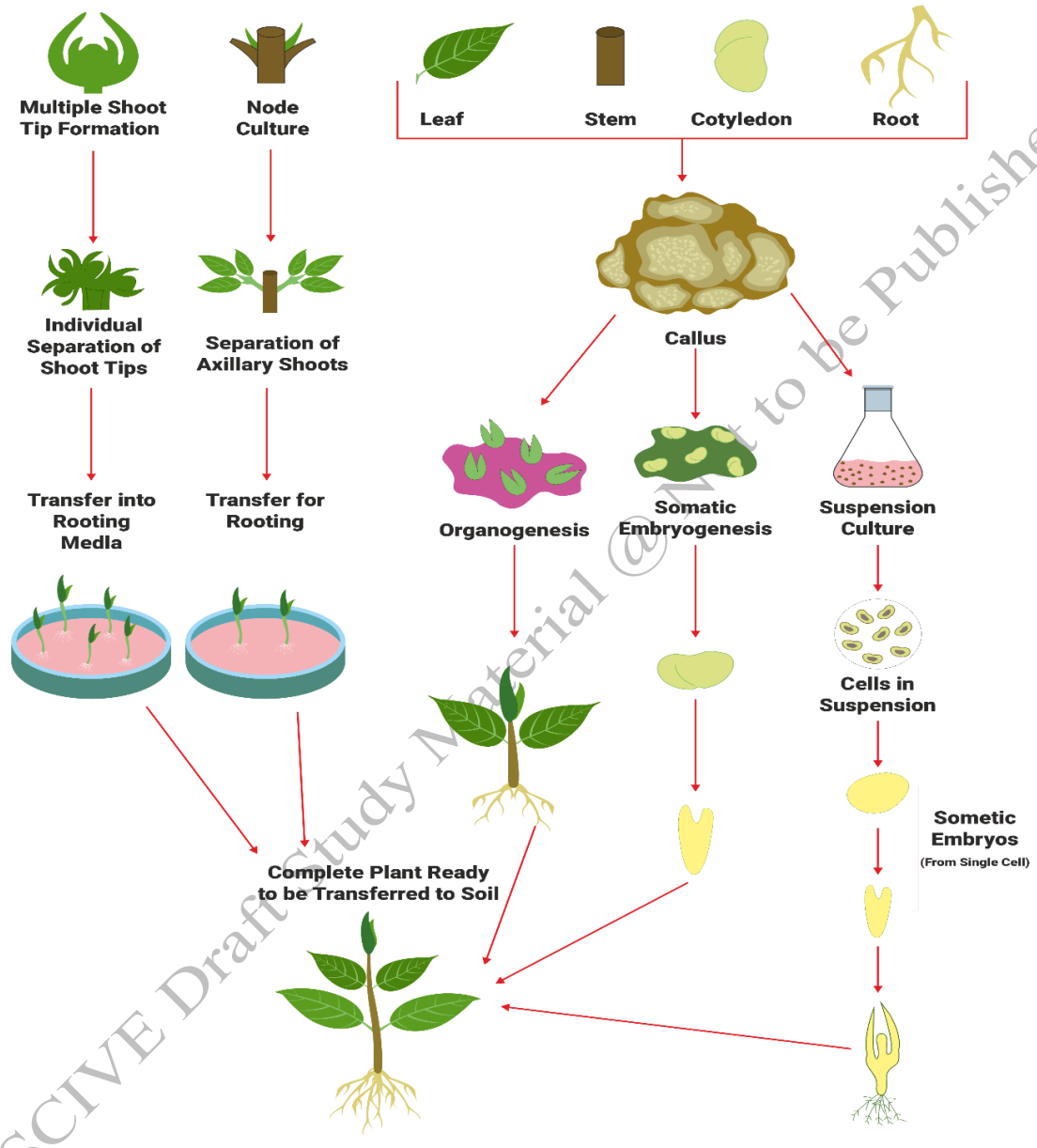

Fig. 2.1: Steps Involved in Organogenesis and Embryogenesis

Process of Excising of explant

The process begins with the selection of a healthy and disease-free mother plant. Commonly used explants such as shoot tips, leaf sections, nodal segments, roots, flowers, etc., are selected. After choosing the plant material, for example, if the nodal segment as explant is chosen, newly emerged shoots are preferred. Once

the plant material is selected, a sterile condition should be maintained at the workplace. All tools, such as scalpels, forceps, and Petri dishes, should be sterilized by autoclaving or surface sterilization with 70% ethanol. Once the selected plant material (shoot) is carefully excised, the collected material should be stored in an icebox and transported to the laboratory before proceeding with the next steps. The plant material is thoroughly washed under running tap water and then immersed in a detergent solution for 5–10 minutes. After treatment, the material is rinsed several times with distilled water. The selected explant is then carefully excised from the cleaned plant material.

Surface Sterilization

Surface sterilization of the explant is the first step in plant tissue culture. The purpose of surface sterilization is to remove contamination from the surface of the excised plant organs. During sterilization it is essential to remove dirt and debris from the explant, and it should be washed in a detergent solution and rinsed several times with distilled water. In case, the explant carries a heavy microbial load, it is advisable to wash it under running tap water for an hour or more. The sterilization procedure can cause damage to the tissue when used directly as an explant source. Therefore, the concentration of the sterilizing agent and the treatment duration should be carefully adjusted to minimise tissue damage. After surface sterilization, the plant material must be rinsed 3-4 times with sterile distilled water to remove all traces of the sterilizing agent. Explants must be treated with an appropriate chemosterilant to eliminate contamination. For each type of explant and plant species, the procedure of surface sterilization should be followed to obtain a high percentage of aseptic and proliferating cultures. While standardising the procedure, the TCDC formula is applied.

Where,

T- Selection of the type of chemical to be used**.**

C- Effective concentration of the selected chemical.

D- The required duration of the treatment.

C- Combination of the treatment if required.

While standardising the procedure, it is important to ensure that the treatment is effective enough to kill the contaminants while its concentration should not be toxic to the proliferating explants.

For leaf: The selected young leaf is surface sterilized. The leaf is dipped into 70% ethanol for 1 minute and then in a 2 % solution of sodium hypochlorite for 20-30 minutes. Then it is washed with sterile distilled water 3-4 times to remove traces of sodium hypochlorite.

Common explant sterilizing agents

Sterilizing agent	Concentration %	Duration' of sterilization (Minutes)
Mercuric chloride	0.01-0.1	0.5-10
Calcium hypochlorite	9-10	5-30
Sodium hypochlorite	2	5-30
Hydrogen peroxide	10-12	5-15
Bromine water	1-2	2-10
Silver nitrate	1	5-30
Silver chloride	0.1-1	2-30

Following chemical sterilization, the explant is rinsed several times with sterile distilled water to remove any residue and then placed on a sterile surface, such as a petri dish, inside the laminar airflow hood. The desired portion of the plant tissue is excised carefully using scalpels. The cuts are made with precision to ensure that the explant remains viable and undamaged. Factors such as the size and shape of the explant are taken into consideration. The excised tissue is immediately transferred to the culture vessel containing the prepared nutrient medium. The placement of the explant is done carefully to ensure proper contact with the medium, which provides the necessary nutrients and hormones for growth and development.

Once the explant is placed in the culture vessel, the container is sealed to prevent contamination. Common sealing methods include the use of parafilm, aluminium foil, or specialised plastic lids. Sealing ensures that the sterile conditions inside the vessel are maintained throughout the incubation period, preventing microbial entry and promoting optimal growth conditions for the

explant. The culture vessels containing the explants are then transferred to a growth chamber with controlled environmental conditions. These conditions include specific temperature settings (e.g., 25±2°C), relative humidity (50–70%), and light intensity (16-hour photoperiod).

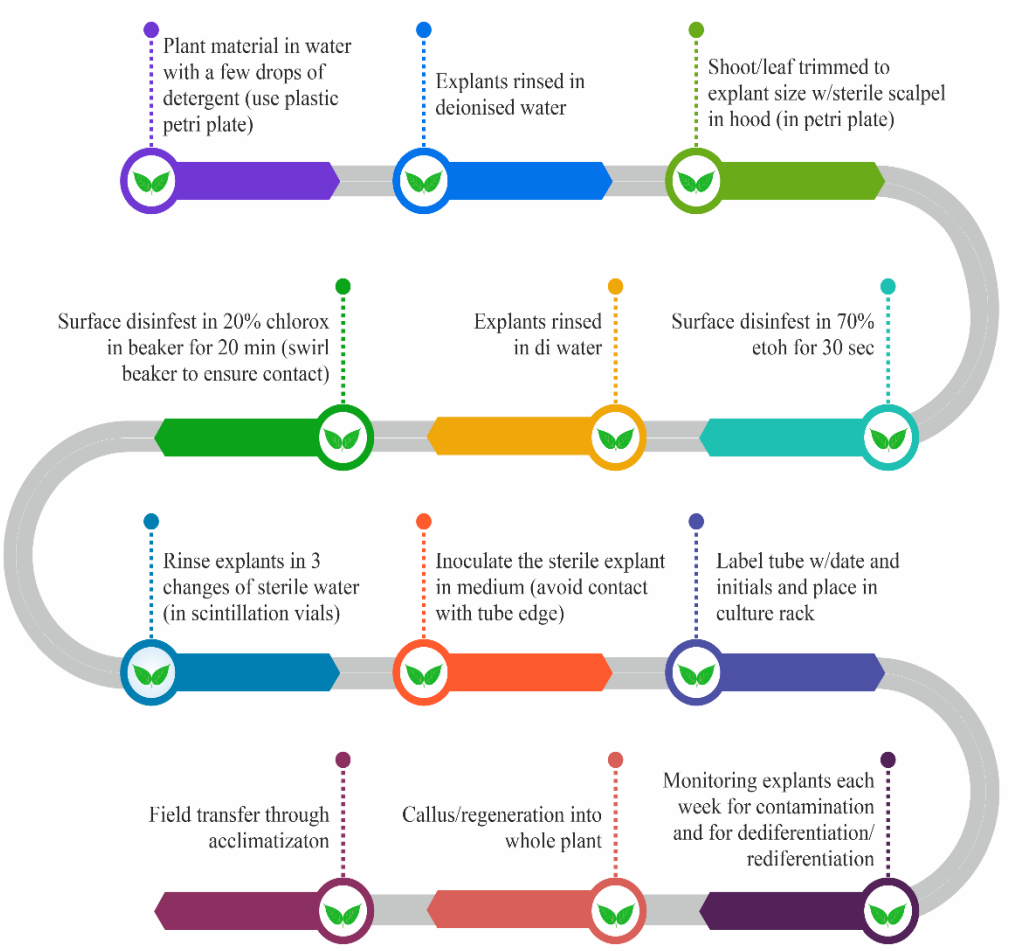

Fig. 2.2: Flow chart depicting the sterilization of explants for tissue culture

Practical Exercises

Activity 1

Demonstrate the techniques involve in the preparation of explants.

Materials required: Pen, pencil, notebook, healthy plant part, beakers and petri dishes, scalpel, forceps, ethanol, bleach solution cotton, distilled water, etc.

Procedure

1. Select a healthy mother plant and choose desired explant (plant part such as leaf, stem, or bud).

2. Wash the selected plant part thoroughly with tap water to remove dirt and dust.
3. Sterilize the plant part by dipping it in ethanol or bleach solution for 2–3 minutes and rinse with distilled water.
4. Use a sterilized scalpel or blade to cut a small piece of the sterilized explant.
5. Place the prepared explant in a sterile petri dish or container for further use in tissue culture.

Check Your Progress

A. Multiple Choice Questions

1. What is an explant?
   a) A nutrient medium
   b) A segment of plant tissue used for *in vitro* culture
   c) A sterilizing agent
   d) A culture vessel
2. Which of the following is a common tool used for excising explants?
   a) Thermometer
   b) Spectrophotometer
   c) Scalpel
   d) Pipette
3. Which of the following sterilizing agents is used at a concentration of 0.1–1%?
   a) Mercuric chloride
   b) Calcium hypochlorite
   c) Sodium hypochlorite
   d) Hydrogen peroxide
4. What is the purpose of sealing culture vessels?
   a) To enhance light intensity
   b) To prevent contamination
   c) To improve sterilization
   d) To increase temperature

5. What is the ideal relative humidity for growth chambers used in tissue culture?
   a) 10–20%
   b) 30–40%
   c) 50–70%
   d) 80–90%

B. Fill in the Blank

1. The primary aim of surface sterilization is to remove _____ from the surface of the explant.
2. _____ is used to sterilize tools, such as scalpels and forceps, for tissue culture.
3. During excision, the explant is transferred to a culture vessel containing a _____ medium.
4. The _____ hood provides a Sterile environment for excising plant tissue.
5. After sterilization, the explant must be rinsed 3-4 times with _____ to remove traces of chemicals.

C. Subjective Questions

1. Explain the importance of surface sterilization in the excision of explants for tissue culture.
2. Describe the process of excising an explant from the mother plant, highlighting the tools and sterilization techniques used.
3. What are the ideal environmental conditions required in a growth chamber for successful tissue culture of explants?

Session 2: Preparing and inoculating an explant to the culture medium

Micropropagation is the *art and science* of multiplying plants under aseptic conditions in an artificial culture medium. It is one of the potent methods of plant tissue culture used by commercial tissue culture laboratories for plant propagation. This technique is commonly applied for the rapid multiplication of new varieties, disease elimination, and cloning of true-to-type plants that cannot be propagated through conventional methods.

Stage of Micropropagation: *In vitro* clonal propagation is a complex process and can be categorised into many stages.

- **Stage 0:** Preparative stage
- **Stage I:** Establishment of aseptic culture
- **Stage II**: Multiplication stage
- **Stage III:** Rooting of shoots
- **Stage IV:** Hardening and Acclimatization

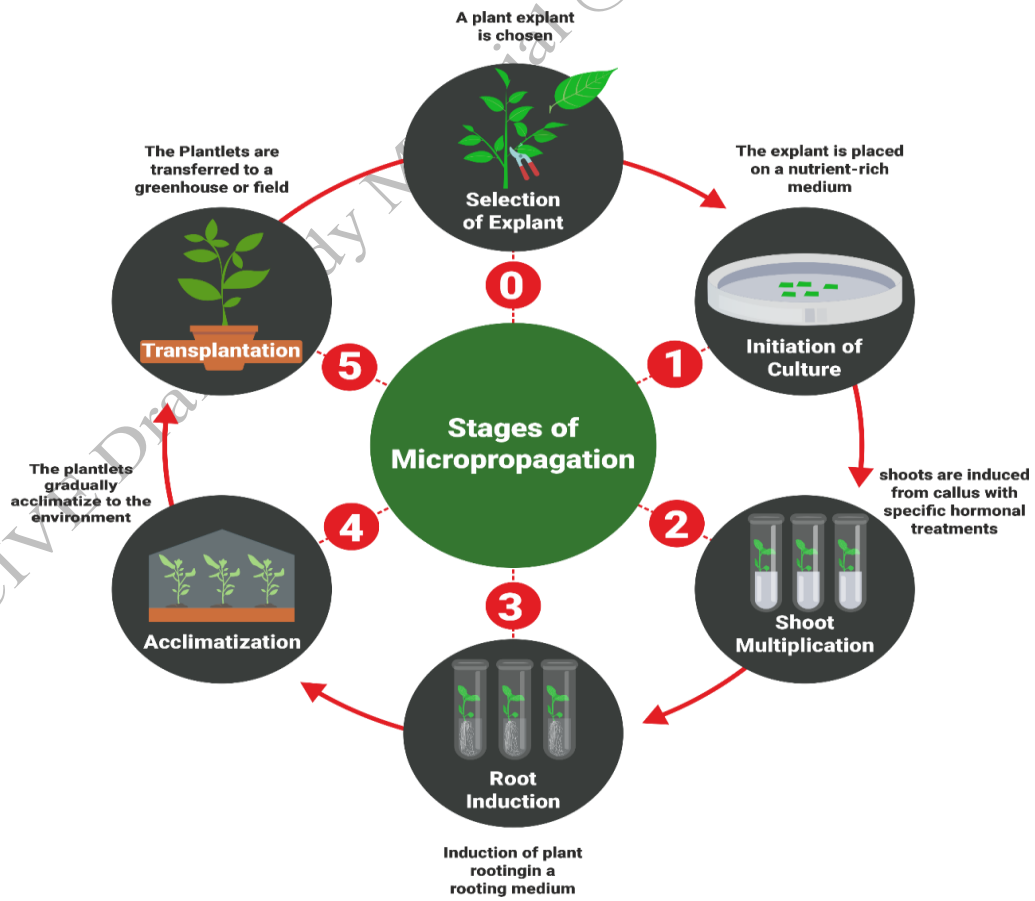

Fig. 2.3: Diagrammatic representation of the stages of Micropropagation

Stage 0: Preparative stage

This stage focuses on preparing mother plants to provide high-quality explants for aseptic culture establishment. To minimise contamination, mother plants are grown under controlled conditions with optimised environmental factors such as light, temperature and growth regulators to enhance the quality of the explant.

Stage 1: Establishment of aseptic cultures

This stage focuses on selecting suitable explants and preparing them for sterile culture to ensure successful propagation. The process of micropropagation starts with the establishment of aseptic cultures. The selection of explants depends upon the pathway of micropropagation and the objective to be achieved. For shoot bud proliferation, nodal segments from sexually mature or seedling material are used. To induce adventitious shoot buds, somatic embryogenesis, or callus, explants such as stem or hypocotyl segments, cotyledon, or leaves are used.

Explants are treated with a suitable chemosterilant to make them free from contamination. As mentioned earlier, for each type of explant and plant species, a specific surface sterilization procedure is followed to achieve a high percentage of aseptic and proliferating cultures.

The choice of explant plays an important role in *In vitro* propagation and depends on the method of shoot multiplication. Explants which carry a pre-formed vegetative bud are suitable for axillary branching. Meristem tip culture may also result in the loss of certain horticultural characteristics the presence of a virus.

Stage 2: Multiplication Stage

This is the most important stage since it is the point where most failures in micropropagation occur. Broadly, three approaches have been followed to achieve *in vitro* multiplication.

i. **Callus culture-mediated approach:** The potentiality of plant cells to multiply indefinitely in cultures and their totipotent nature allows a rapid multiplication of several plant types. Differentiation of plants from cultured cells may occur via organogenesis or somatic embryogenesis. Somatic embryogenesis is particularly attractive from a commercial perspective because, once established, it offers better control than organogenesis.

ii. **Adventitious bud formation:** Adventitious buds are those that arise from locations other than the leaf axil or the shoot apex. Shoots differentiated from calli are also considered adventitious buds. In many horticultural crops, vegetative propagation through adventitious bud formation from roots (e.g., blackberry, raspberry) and leaves (e.g., Begonia, Crassula) is standard horticultural practice. Under culture conditions, the rate of

adventitious bud development can be significantly enhanced. For most bulbous plants (e.g. Lily), adventitious bud formation from bulb scale explants is the primary method of multiplication.

iii. **Enhanced axillary branching:** In cultures, the rate of shoot multiplication by axillary branching can be substantially enhanced by growing shoots in a medium containing a suitable cytokinin at an appropriate concentration with or without auxin. Due to the continuous availability of cytokinin, the shoots formed by the bud present on the explant develop axillary buds which may grow directly into shoots. This process may be repeated several times, and the initial explant transformed into a mass of branches.

Somatic embryos have a pre-formed radicle and may develop directly into plantlets. However, these embryos often show very poor conversion into plantlets, particularly under *In vitro* conditions. To achieve normal germination, these embryos require an additional maturation step. Adventitious and axillary shoots developed in cultures with cytokinin generally lack roots. To obtain full plants, the shoots must be transferred to a rooting medium which differs from the shoot multiplication medium, particularly in its hormonal and salt compositions. For rooting, individual shoots measuring around 2 cm in length are excised and transferred to the rooting medium.

Stage 3. Rooting of Shoots

In this phase, shoots of appropriate size are excised from the multiplying shoot culture and inoculated on a medium containing root-inducing hormone. Auxins such as Indole-3-butyric acid [IBA], Indole-3-acetic acid [IAA], Naphthalene acetic acid [NAA] are generally used in various concentrations and combinations to induce roots *In vitro* developed shoots.

Individual micro-shoots or bunches of micro-shoots (each containing 5-6 shoots with an average length of 4.0 cm and a diameter of 2.0 mm) can be transferred to a half-strength MS liquid medium containing 10 µM IBA and 3% sucrose. These are incubated in the dark for 1 week. After a week, the medium is replaced by MS liquid medium without Plant Growth Regulators (PGRs) but still containing 3% sucrose. The survival rate of rooted cultures is highest when they are maintained for 6 weeks before being transferred to a greenhouse for hardening. Once a good root system develops (after 3 weeks), the plants are transferred to 6-inch plastic pots containing a sterile mixture of sand, soil, and cow-dung manure in a ratio of 1:1:1 (w/w/w). The pots are then kept in the greenhouse for acclimatization.

Stage 4. Hardening and Acclimatization

The ultimate success of commercial propagation largely depends on the ability to transfer plants out of culture on a large scale at low cost and with high survival rates. Therefore, gradual acclimatization is necessary for these plants to survive the transition from culture to the greenhouse or field environment. Hardening is a process in which tissue culture plants are gradually acclimatized to overcome physiological disorders they develop in *In vitro* culture. Hardening and Acclimatization are the most critical steps to achieve success in micropropagation. Plants grown in tissue culture are adapted to the controlled laboratory environment in a culture room where the light intensity is low compared to the external environment and temperatures are consistently maintained around 25-28ºC. In the beginning, the plants, after their removal from the rooting medium, are transferred to culture bottles containing soilrite, which is moistened with a nutrient solution of reduced salt concentration and kept closed in a culture room in an aseptic environment. By doing so, the tissue culture plants become normal. Such bottles are subsequently shifted to a greenhouse environment, which maintains a low humidity (60-70%) and temperature 28-30˚C and light intensity. During acclimatization, the *In vitro* formed leaves do not recover, but the plant develops normal leaves and functional roots. For the initial 10-15 days, it is essential to maintain high humidity (90-100%) around the plants, to which they have adapted during culture. The humidity is gradually reduced to ambient level over 2-4 weeks, allowing the plants to fully acclimatize.

Elimination of viruses

Elimination of viruses or development of virus-free propagules can also be achieved using meristem culture, which is part of the rapid micropropagation cycle. The process typically involves culturing the meristematic tissue. which is likely to carry viral infections. Recently, cryotherapy has emerged as a new method for virus elimination from tissue-cultured meristem and shoot tips of various plant species.

Practical Exercises

Activity 1

Demonstrate steps of explant inoculation.

Materials required: Pen, pencil, notebook, sterilized explants, nutrient culture medium, scalpel forceps, sterile gloves, apron, laminar airflow chamber, ethanol (70%), distilled water, beakers, cotton swabs, etc.

Procedure:

1. Clean the laminar airflow chamber and wipe the surface with ethanol.
2. Sterilize tools like scalpel and forceps and sanitize hands with ethanol.
3. Cut the sterilized explant into small pieces using the scalpel.
4. Place the explant gently on the nutrient medium in the test tube inside the airflow chamber.
5. Close the test tube tightly with cotton swab to prevent contamination
6. Label it with details like plant type and date of inoculation, etc.
7. Keep the test tube in a controlled environment condition for growth.

Activity 2

Demonstrate the rooting process in plantlets.

Materials required: Healthy plantlets (shoots), rooting hormone, rooting medium (sterile soil, coco peat, or agar), scalpel or sharp blade, forceps, beakers or pots, sterile water, gloves, apron, etc.

Procedure:

1. Select healthy plantlets and trim the cut end of the shoots.
2. Dip the cut end into rooting hormone.
3. Prepare the rooting medium in pots or beakers.
4. Insert the cut end of the plantlet into the rooting medium.
5. Cover the pots with a plastic dome or place them in a humidity chamber.
6. Observe the rooting success.

Check Your Progress

A. Multiple Choice Questions

1. Which stage in micropropagation involves the establishment of aseptic cultures?
   a) Stage 0
   b) Stage I
   c) Stage III
   d) Stage IV
2. What type of explant is most suitable for somatic embryogenesis?

a) Nodal segment
b) Meristem tip
c) Stem or cotyledon segments
d) Whole plant

3. Which hormone is primarily used for root induction during Stage III of micropropagation?
   a) Cytokinin
   b) Auxin
   c) Gibberellin
   d) Abscisic acid
4. Which medium is commonly used for micropropagation?
   a) MS medium
   b) PDA medium
   c) LB medium
   d) EMB medium
5. What is the optimal temperature range for tissue culture room conditions?
   a) 15-20°C
   b) 25-28°C
   c) 30-35°C
   d) 40-45°C

B. Fill in the Blank

1. The preparative stage of micropropagation is referred to as _____.
2. _____ culture is a technique used in micropropagation to eliminate viruses from plants.
3. Rooting of micropropagated shoots is achieved by using auxins like _____ and NAA.
4. In Stage IV of micropropagation, plants are transferred to greenhouse for _____.

C. Match the followings

Column A	Column B
1- Stage 0	a) Induction of roots in shoots using auxins
2- Stage I	b) Transition of plants to external environment
3- Stage II	c) Preparation of mother plants for explant collection
4- Stage III	d) Rapid shoot proliferation through axillary branching

D. Subjective Questions

1. Explain the role of cytokinin in the multiplication stage of micropropagation.
2. What is the importance of hardening and acclimatization in micropropagation?
3. List the different stages of micropropagation and briefly describe their purpose.

Module 3
Acclimatizing the Tissue Cultured Plants

Module Overview

Tissue culture is an advanced plant propagation technique in plant biotechnology that enables the growth of entire plants from small pieces of plant tissue. This method is used to produce large numbers of identical plants in controlled laboratory environments. These plants are grown in nutrient-rich medium under sterile conditions. This technique is especially important for plants that are difficult to propagate through traditional methods or for producing disease-free plants in large quantities. However, the process doesn't end till a fully formed plantlet is produced in the culture vessel. The final and crucial step is acclimatization which prepares the tissue-cultured plants for survival in the outside environment. However, once these tissue-cultured plants are ready, they cannot be moved directly to open fields or greenhouses because the conditions outside the lab such as light, temperature, and humidity are different. Acclimatization help the plants to gradually adapt to these environmental changes. During acclimatization, plants are gradually exposed to the natural environment to ensure their survival and growth.

This module provides students with an in-depth understanding of the acclimatization process. Session 1 covers the techniques and stages involved in hardening, including gradual exposure to external factors such as light, temperature, and humidity. Session 2 discusses the types of crops best suited for tissue culture and emphasizes efficient use of resources for optimal growth and productivity.

Learning Outcomes

After completing this module, you will be able to:

- Explain the process of acclimatization (hardening) of tissue-cultured plants and its significance in ensuring plant survival under natural conditions.
- Identify the types of crops suitable for tissue culture and understand how to optimize resources for efficient large-scale propagation.

Module Structure

- Session 1: Process of Acclimatization (Hardening)
- Session 2: Explain types of crops suitable for Tissue culture and resource optimization

Session 1: Process of Acclimatization (Hardening)

Acclimatization refers to the ability of *In vitro* plants to adapt to a new environment. Tissue culture plants are subjected to both biotic and abiotic challenges when they are moved from a lab setting to the soil. Abiotic stresses include changes in temperature, light intensity, and humidity, while biotic stresses include the presence of soil microflora (microbes living in soil). Hence, tissue culture plants require a gradual step-wise acclimatization to successfully establish themselves in the natural environment.

Purpose of Acclimatization

The primary purpose of acclimatization is to help tissue-cultured plants, which have been nurtured in a controlled laboratory environment, adjust to the more variable and often harsh conditions of the outside world. These plants are too sensitive to withstand any sudden changes that could take place outside. The main purpose of acclimatization is to assist plants in transitioning from externally supplied sugars to self-sufficient food production (heterotrophic to autotrophic). There are several reasons why acclimatization is required, such as:

a. *In vitro* regenerated plants are initially heterotrophic, meaning they rely on external sources of nutrition. Acclimatization helps convert these plants into autotrophic ones, allowing them to produce their own food through photosynthesis, making them capable of independent growth.

b. *In vitro* conditions maintain constant temperature, light levels, and humidity, but these variables fluctuate in the natural environment. Acclimatization allows plants to gradually adjust to these changing conditions, ensuring their survival in the external environment.

c. Tissue-cultured plants are grown in sterile conditions and hence are not exposed to diseases or pests. Acclimatization helps them acquire natural resistance by exposing them gradually to the external environment.

d. *In vitro* plants, usually grown in nutrient medium or liquids, develop shallow root systems. Acclimatization helps plants develop strong and extensive root systems that allow them to absorb nutrients and water from the soil.

Tissue-cultured plants may experience environmental shock in the absence of adequate acclimatization, which might result in stunted growth or even death. Therefore, proper steps of acclimatization should be followed.

Steps of Acclimatization

The acclimatization process is divided into two stages: primary and secondary. These stages gradually adjust the plants to the external environment through a series of steps. If the roots of the plants are well developed, then there is no need to transfer the plant to a nutrient solution, and it can be directly transferred to the greenhouse.

A-Primary Acclimatization

I. **Transfer the plant to the nutrient solution:** Tissue-cultured plants are grown in sterile containers containing nutrient-rich media. Once the plants have reached a height of 3-5 cm and have well-developed roots, they are carefully removed from the culture vessels. It is important to handle the plants gently, as they are delicate and fragile and can be easily damaged. Use sterilized forceps or tweezers to extract each plantlet without breaking roots or stems. Gently wash off any culture medium from the roots. Transfer plants to small pots with sterile, airy and well-draining growing medium. A growing medium or nutrient solution with half the usual concentration is used to support the growth and development of plantlets. The half-strength Hoagland nutrient solution promotes stronger root growth by limiting the availability of inorganic nutrients. The reduced nutrient concentration encourages the plantlets to produce a strong root system, enabling them to absorb more nutrients from the soil. This process enhances the nutrient uptake and photosynthetic ability of plants, leading to greater overall stability and resilience due to the absence of organic nutrients/carbon source in the Hoagland nutrient solution.

II. **Transfer to Greenhouse**: Transferring the tissue-cultured plants from the laboratory to a greenhouse is the first stage of acclimatization. Greenhouse provides a semi-controlled environment, offering more flexibility than a lab while still protecting the plants from adverse weather conditions. The environmental conditions in a greenhouse differ slightly from those in the lab, with higher temperatures and lower humidity. Gradually increasing the light and temperature while decreasing the humidity helps the plants adapt slowly to these new conditions, ensuring a smooth transition from the controlled laboratory environment to the more variable conditions outside.

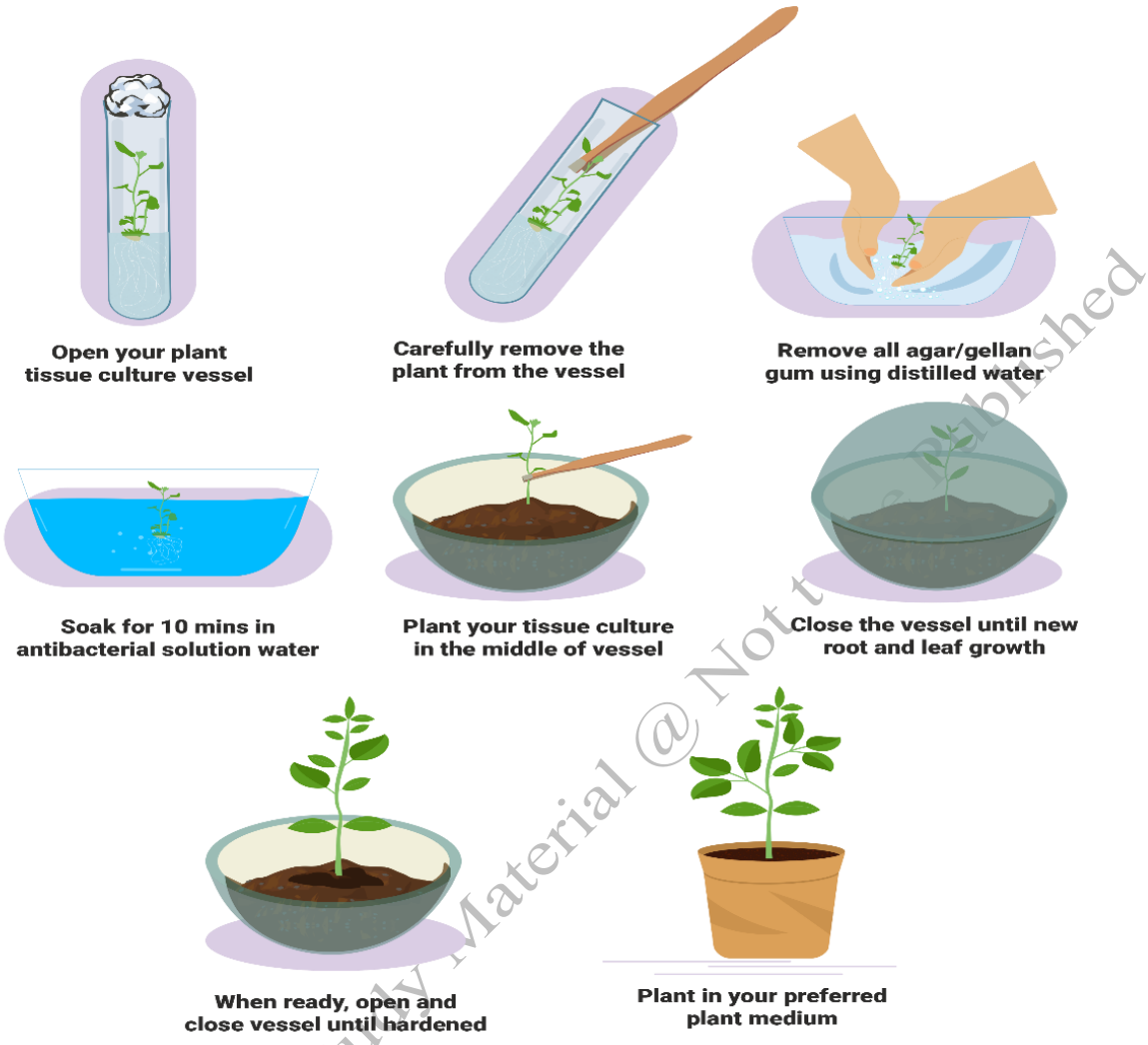

Fig. 3.1: Diagrammatic representation of Acclimatization

B- Secondary Acclimatization

Once the plants have acclimated to the greenhouse environment, they are transferred to soil-filled pots. At this stage, as the plants begin to receive nutrients from the soil instead of the nutrition medium utilised in the lab, it promotes the development of strong, well-established roots. The final stage of secondary acclimatization involves gradually exposing the plants to outside conditions for short periods, allowing them to experience wind, direct sunlight, and temperature fluctuations. Over a few weeks, the duration of exposure is progressively increased until the plants are fully prepared to survive outside year-round. This step is essential for helping tissue-cultured plants transition from their controlled *In vitro* environment to *ex vitro* conditions, where they must independently regulate water loss and gas exchange. Effective acclimatization

protocols include gradual adjustments in humidity and light, careful monitoring of plant water status, and in some cases, the use of antitranspirant agents to reduce excessive transpiration.

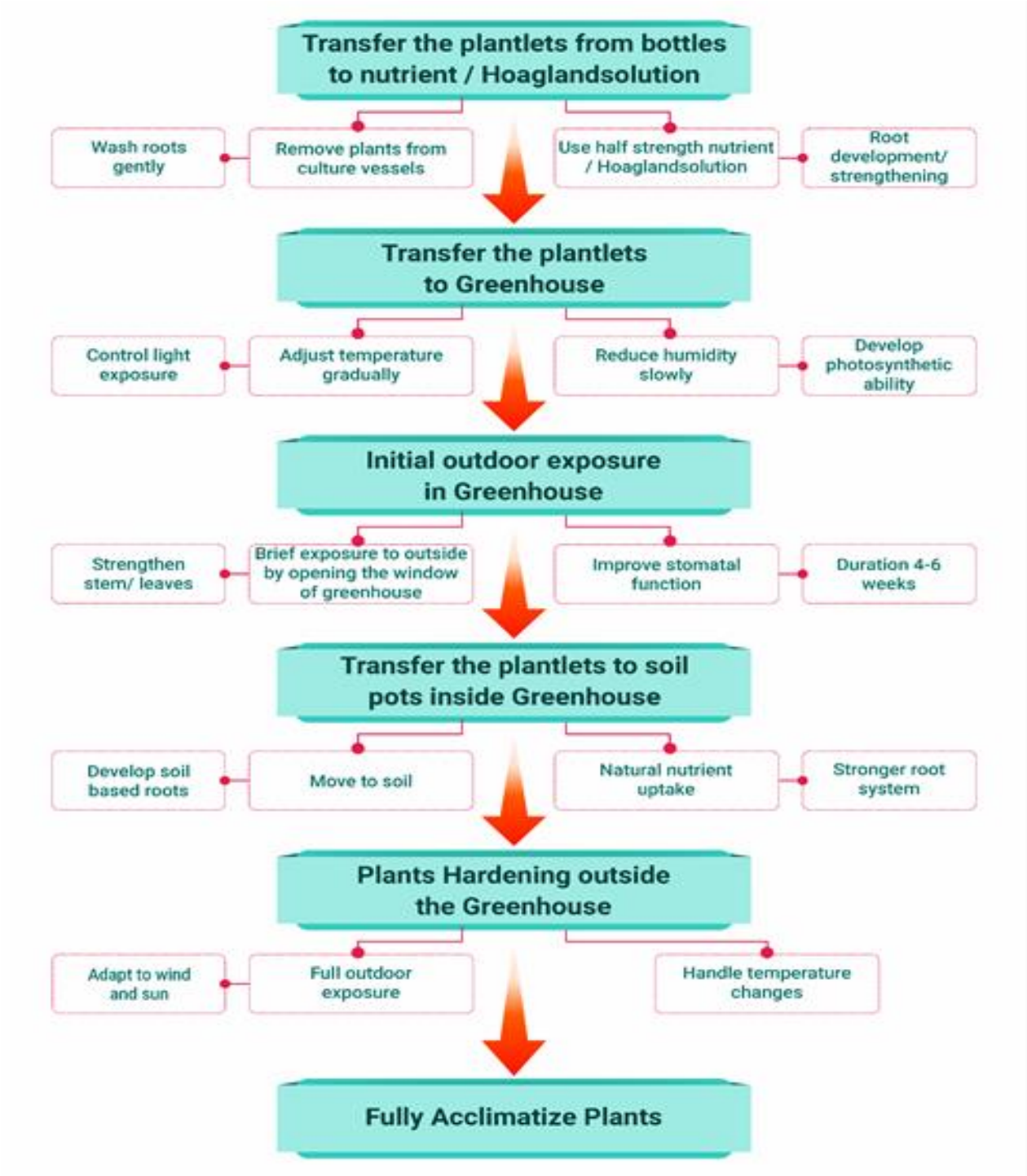

Fig. 3.2: Steps involve in Acclimatization

Precautions during the Acclimatization of cultured plants

Tissue culture plants often face several challenges when transferred to natural conditions. Their root systems are not well-developed, which makes it difficult for the plants to absorb sufficient water. As a result, they are prone to dehydration due to poor regulation of water loss.

Light exposure can negatively affect their growth. When these plants are suddenly exposed to high-intensity light, it can damage the chloroplasts, reducing their photosynthetic efficiency and hindering their overall growth.

In tissue culture conditions, plants grow in nutrient-rich media where water and nutrients are readily available, so they do not need to struggle for resources. However, this results in a weak nutrient uptake mechanism. When transferred to soil, the underdeveloped roots find it difficult to absorb nutrients and adapt to the new environment.

Tissue culture plants are initially grown under sterile lab conditions and hence are not exposed to microorganisms. When transferred to soil, they encounter a variety of microbes, which can stress their limited immunity and tolerance to external conditions.

In vitro plants grow under stable controlled conditions such as light, humidity, and temperature. Sudden changes in these factors after transfer to the outside environment can cause stress and hinder plant adaptation.

Appropriate conditions for transplanting acclimatized plants

Location for transplanting that receives plenty of sunlight, has well-drained soil, and is free from competition with weeds should be preferred. Transplanting should be done in the early morning or late afternoon to avoid heat stress. The crop's growth season should match the transplanting time to ensure optimal growth. Immediately after transplanting, plants should be watered generously. During the first few days, the soil should be kept lightly moist till the roots settle. To protect young plants from pests, wind, and direct sunlight in the early stages, shade netting or coverings should be used to create a conducive environment.

Successful transplanting of tissue-cultured plants from the lab or greenhouse to open fields requires careful preparation and management. The soil should be free of pathogens, rich in organic matter, and well-drained, with a pH between 6.0 and 7.0 for most crops. To improve fertility, fertilizers or compost can be added if necessary. Planting should be done when the ambient temperature suits the crop

requirements, ideally between 18°C - 25°C. Extreme temperatures, whether too high or too low, should be avoided as they can stress the plants and slow their growth.

Water management is another crucial factor. The soil must remain moist but not waterlogged to avoid root damage. Gradual exposure to sunlight is also essential. Tissue-cultured plants, which are initially grown in controlled environments, need time to adapt. Hence, begin with partial shade and slowly increase their exposure to sunlight to prevent sunburn or wilting.

Young plants are particularly vulnerable to pests and diseases, so regular monitoring is necessary. Organic pest management techniques can help maintain plant health without harmful chemicals. Proper spacing of plants ensures they receive adequate nutrients, water, and sunlight. For climbing or tall-growing plants, support structures like trellises or stakes should be used to minimise damage during growth. By following these steps, tissue-cultured plants can successfully adapt to outdoor conditions and thrive in open fields or larger greenhouses.

Practical Exercises

Activity 1

Demonstrate hardening process of tissue cultured plant.

Materials required: Pen, pencil, notebook, etc.

Procedure:

1. Carefully remove the plantlets from the culture vessels, ensuring that the roots remain intact.
2. Select the tissue cultured plant and place them gently in hardening chamber/greenhouse under supervision of teacher.
3. Irrigate the plants to ensure proper moisture conservation and hydration.
4. Monitor the temperature and humidity levels during the hardening process.
5. Regularly monitor the health of the plants.
6. Observe the plant on regular basis.

Activity 2

Demonstrate the method of transplanting tissue cultured plants.

Materials required: Tissue-cultured plant, small pots or trays, sterile soil, watering can, scalpel or sharp blade, gloves, etc.

Procedure:

1. Select tissue cultured plant from hardening chamber/greenhouse
2. Fill small pots or trays with growing media.
3. Place the plantlet in the growing media and cover the roots.
4. Gently water the plants.
5. Place the transplanted plant in a partially shaded condition for few days.

Check Your Progress

A. Multiple Choice Questions

1. What is the main purpose of acclimatization in tissue-cultured plants?
   a) To increase plant height
   b) To help plants transition to external environments
   c) To eliminate pests
   d) To enhance flowering
2. Which of the following is a common abiotic stress during acclimatization?
   a) Pests
   b) Microbial attacks
   c) Temperature changes
   d) Fungal infections
3. Which solution is used for root development in primary acclimatization?
   a) Hoagland solution
   b) Nutrient gel
   c) Agar medium
   d) Compost tea
4. In secondary acclimatization, plants are transferred to:
   a) Greenhouse conditions
   b) Open-field conditions
   c) Soil-filled pots
   d) Nutrient-rich culture vessels

5. What is the ideal pH range of soil for transplanting tissue-cultured plants?
   a) 4.5-5.0
   b) 5.5-6.0
   c) 7.5-8.0
   d) 6.0-7.0

B. Fill in the Blank

1. The primary purpose of acclimatization is to help plants adapt to _____ and _____ stresses.
2. During primary acclimatization, plants are transferred to a _____ with half the usual concentration.
3. The _____ solution is commonly used during primary acclimatization to promote stronger root growth.
4. Plants are more likely to experience environmental _____ without proper acclimatization.

C. Subjective Questions

1. Why is acclimatization essential for tissue-cultured plants?
2. Briefly describe the steps of primary acclimatization.

Session 2: Types of Crops Suitable for Tissue Culture and Resource Optimization

Crops Suitable for Propagation by Plant Tissue Culture

Plant tissue culture has a wide range of applications in agriculture, horticulture, and conservation. The choice of plant depends on the elite characteristics of the plant material and the benefits that tissue culture can provide, such as faster growth, disease resistance, and the ability to produce large numbers of plants. The following important crops can be multiplied by tissue culture:

1- **Ornamental Plants**: Plant tissue culture plays an important role in floriculture sectors. Ornamental plants are grown mainly for aesthetic reasons and decorative purposes. Some of the well-established examples of ornamental plants are Roses, Chrysanthemums, Orchids, Gerberas and Carnations. Tissue culture is widely used to reproduce ornamental plants due to their high market value, steady demand and requirement for disease-free plants. Naturally, orchids propagate slowly, but tissue culture enables the rapid multiplication of rare and valuable varieties.

2- **Fruit Plants**: Fruit crops have a significant commercial value and are vital for nutrition. Their large-scale production and improvement are greatly enhanced by tissue culture techniques. Tissue culture is important for many fruit crops, particularly those that are vegetatively propagated. Banana is the most popular fruit crop commercially propagated by the tissue culture method. Many vegetatively propagated fruit crops also benefit from this technology. Tissue culture permits the production of disease-free planting material and enables the rapid multiplication of new and improved varieties. **Examples:** Bananas, Pineapples, Strawberries, Apples, Grapes.

3- **Vegetable Crops**: Vegetables are an important component of world agriculture and are essential to human nutrition. In vegetable crops, tissue culture is very helpful for creating planting material free of disease, preserving germplasm, and developing improved varieties through somaclonal variation. **Examples:** Asparagus, Cauliflower, Carrots, Potato, Tomato

4- **Forestry species**: Forest trees are essential for the economy and ecology because they provide important goods like pulp, timber, and other resources. However, complex reproductive processes and lengthy generation durations are two issues that tree species frequently encounter.

Tissue culture provides an answer by helping to conserve rare or endangered species and enabling the quick multiplication of exceptional genotypes. **Examples:** Poplar, Pine, Teak, Rubber, Eucalyptus

5- **Medicinal Plants**: Medicinal plants are essential to traditional medical systems and provide pharmaceutical companies with valuable secondary metabolites. Many therapeutic plants are slow-growing, difficult to propagate, or in danger of going extinct as a result of overuse in their native environments. Tissue culture offers a solution by enabling the preservation of these species and the production of plants with consistent therapeutic properties, ensuring their continued availability for medicinal use. **Examples:** *Aloe vera, Stevia, Digitalis*, Ashwagandha

6- **Spices**: In addition to adding flavour and aroma to food, spices and flavouring compounds also have therapeutic benefits. Many spice crops are susceptible to diseases and difficult to cultivate using traditional methods. Tissue culture aids in the preservation of genetic resources and the production of disease-free planting material. **Examples:** Cardamom, Vanilla, Black Pepper, Turmeric

7- **Cash Crops**: Cash crops, which are vital to the economies of many nations, are agricultural products grown primarily for commercial purposes. Cash crops frequently need homogeneous, large-scale planting material. The generation of disease-free plants and the quick replication of superior types are made possible via tissue culture. **Examples:** Cotton, Tea, Coffee, Sugarcane

Resource Optimisation

Resource optimization is a critical concept in modern scientific and industrial practices, focusing on maximising efficiency while minimising waste. It involves the strategic use of available resources, including materials, energy, time, and human capital, to achieve optimal outcomes. Resource optimisation means using available resources in the most efficient way possible, which leads to many benefits such as cost reduction, improved productivity, and reduced environmental impact.

Benefits of Resource Optimisation

- Using resources efficiently can help organisations reduce operational costs. For example, a plant tissue culture lab that optimises its growth media components can reduce the cost per plant by 20-30%. Using fewer or

alternative natural resources like energy, water, and chemicals lowers expenses, which can be redirected toward purchasing affordable products or increasing research funding.

- Efficient use of resources reduces waste and pollution, which benefits the environment. Using less energy, for instance, helps lower greenhouse gas emissions and minimises the impact on the environment.

- Optimised processes allow organisations to do more with the same or fewer inputs. For example, an improved plant tissue culture protocol can increase the number of viable plantlets produced per batch by 50% without any additional space or equipment. This increased productivity accelerates research or boosts commercial production capacity.

- Better processes often lead to consistent and higher-quality results. In plant tissue culture, this means healthier plantlets with better survival rates during acclimatization. Plants also grow better in an optimised growth medium, which leads to more reliable research outcomes or higher-value commercial products.

- By minimising resource wastage and using materials efficiently, resources can be conserved for future use. Better planning also reduces waste, which is good for the environment and saves on disposal costs.

Process of Resource Optimisation in a Plant Tissue Culture Lab

In a plant tissue culture lab, optimising resources ensures both cost savings and efficiency. In the lab, the first step in resource optimisation is to measure how much energy, water, and growth media are being used. Water should be used carefully, and recycling should be done when possible, such as reusing water from cooling systems. Culture media should be prepared in the exact quantity to avoid wastage, and any unused media should be stored properly for future use. Whenever possible, equipment should be reused. Items such as glass bottles and culture tubes can be sterilized and reused, reducing the need for new supplies. To reduce environmental impact, the use of single-use plastics should be minimised, instead, reusable or biodegradable alternatives should be used when possible. Additionally, staff should be trained to use resources efficiently. Teaching staff about resource optimisation and encouraging them to suggest improvements can help increase efficiency. Regular monitoring ensures that resource-saving practices are working. It is important to keep records and analyse data to find areas where further improvements can be made.

Methods of Recycling and Disposing of Waste

Labs produce various types of waste. Waste management is important for a lab to operate sustainably. In the lab, glassware should be cleaned and sterilized for reuse. Autoclavable glassware is ideal, as it can be sterilized using an autoclave, which employs steam under pressure. It is important to clean the glassware with appropriate detergents and to avoid using any damaged glassware to prevent injury. Similarly, chemicals used in the lab should be handled carefully. Some chemicals can be recycled, breaking down complex substances like polymeric waste into raw materials for other products. Other chemicals must be disposed of properly, following safety protocols to prevent environmental damage. Chemicals should be categorised based on their properties, such as organic solvents, acids, or bases, and some toxic chemicals can be neutralised through specific chemical reactions.

Organic waste, such as plant debris, can be composted to create fertilizer. Uncontaminated plant matter should be collected, mixed with other organic materials, and allowed to break down. The resulting compost can be used to fertilize non-lab plants. Water used in lab processes can also be recycled to reduce consumption. Wastewater can be collected, purified through filters and other methods, and then reused for tasks like watering plants or cleaning. This approach helps conserve resources and minimise environmental impact.

Practical Exercises

Activity 1

Demonstrate the process of recycling and disposing different types of waste.

Materials required: Plastic bottles, paper, cardboard, glass jars, food scraps, biodegradable and non-biodegradable bags, gloves, trash bins, compost bin, recycling bins, pen, pencil, notebook, etc.

Procedure:

1. Collect different types of waste such as plastic, paper, glass, and food scraps.
2. Sort the waste into two categories: biodegradable (organic) waste (like food scraps) and non-biodegradable waste (like plastic, paper, and glass).
3. Place the biodegradable waste in a compost bin.
4. Clean the plastic bottles, glass jars, and cardboard and place them in separate recycling bins.
5. Dispose off non-recyclable waste like plastic bags in the trash bin.

Check Your Progress

A. Multiple Choice Questions

1. Which of the following crops is NOT typically propagated using tissue culture?
   a) Orchids
   b) Bananas
   c) Wheat
   d) Pineapples
2. What type of crop is Aloe vera classified as?
   a) Fruit crop
   b) Medicinal plant
   c) Cash crop
   d) Ornamental plant
3. Which is a key benefit of resource optimization in tissue culture labs?
   a) Higher costs
   b) Increased waste
   c) Reduced operational costs
   d) Decreased productivity
4. Which of the following materials can be sterilized and reused in tissue culture labs?
   a) Plastic containers
   b) Glassware
   c) Paper towels
   d) Disposable gloves

B. Fill in the Blank

1. The most popular fruit crop commercially propagated through tissue culture is _____.
2. Tissue culture helps produce planting material free from _____.
3. _____ are used as reusable alternatives to single-use plastics in tissue culture labs.

C. Subjective Questions

1. What are the advantages of resource optimization in tissue culture labs?
2. Why is tissue culture important for the rapid multiplication of ornamental plants?

Module 4
Hygiene, Cleanliness, Safety and Emergency Procedures at Workplace

Module Overview

Different workplaces have different levels of challenges, especially in terms of physical hazards inherent like work or the workplace. Workplace accidents sometimes result in harmful, unfortunate, and counter-productive impacts on workers, co-workers, and their families. They suffer pain, disability, stress, and in some cases even loss of employment. A Hazard is therefore defined as a condition or event that portends or has the potential to cause injury, threaten life, damage property, etc. Identifying and managing hazards in the workplace is crucial for maintaining a safe environment, protecting the well-being of employees, and ensuring the overall productivity and efficiency of the organisation. Effective hazard identification, risk assessment, and implementation of safety protocols are essential to minimise the chances of accidents and their negative impacts.

Hazards in tissue culture labs include mechanical hazards, chemical hazards, accidents, hazards related to the occupancy of confined places, occupational diseases, and other related hazards. It is essential to prioritise the personal safety of workersBased on ethical, health, and professional standards. This unit will help you learn about various health and physical hazards faced by workers and the safe work procedures that ought to be adopted for reducing the persisting risks and preventing the occurrence of accidents.

This module emphasizes the importance of personal safety, emergency preparedness, and proper handling of laboratory tools. In Session 1, students will learn about the use of first-aid kits, basic treatment procedures for minor injuries, and the correct usage of safety equipment to respond to emergencies effectively. Session 2 focuses on the safe handling and operation of laboratory equipment, including guidelines to prevent accidents and ensure a clean, organized working space.

Learning Outcomes

After completing this module, you will be able to:

- Identify the key first-aid treatments and safety equipment required in a laboratory setting, ensuring preparedness for emergencies.

- Understand the safe use and handling of laboratory equipment, adhering to best practices for maintaining a safe work environment.

Module Structure

- Session 1: First-Aid, Treatment and Safety Equipment
- Session 2: Safe Use of Lab Equipment

Session 1: First-Aid, Treatment and Safety Equipment

Accidents might happen in spite of all the precautions and care. It is essential for students to know about the immediate medical aid for a chemical accident and to learn about the safety devices needed to prevent accidents.

Common Health and Safety guidelines followed in the workplace

Maintaining a safe and efficient laboratory environment is crucial to protect researchers from potential hazards posed by chemicals, equipment, and other agents used in plant tissue culture. Adhering to the guidelines given below ensures both safety and the integrity of experiments:

- Always read instructions and warning signs before using any equipment or chemicals.
- Be aware of the location and use of safety kits, equipment such as eye wash solutions, first aid kits, and fire extinguishers.
- Wear lab coats, gloves, protect eyes, and wear masks while handling chemicals.
- Protect yourself from exposure due to UV light or other hazardous materials.
- Use fume hoods when working with volatile or hazardous chemicals to prevent inhalation of dangerous fumes.
- While diluting acids, always add acid to water slowly to avoid splattering. Handle acids, caustic materials, and strong oxidizers in a sink for safe containment.
- In case of injury or accidental chemical exposure, seek medical attention immediately.
- Properly label all chemical containers and dispose of expired chemicals according to safety protocols, ensuring safe disposal of radioactive or hazardous waste.
- While handling bottles, grip them securely around the body, and not by the neck, to prevent spills and accidents.

- Do not eat, drink, smoke, or apply cosmetics in the lab. Maintain good hygiene, keep short nails, tie back long hair and avoid loose clothing or jewellery.
- Clean and disinfect work surfaces and benches at the end of each day to maintain a contamination-free lab environment.
- Familiarise yourself with the operation of all safety devices in the lab and ensure easy access to them at all times.

There are certain chemicals which are hazardous and should be taken care of. They can be categorised as flammables, combustibles, explosives, oxidatives, toxic materials, compressed gases, corrosive materials, irritants and carcinogens.

General housekeeping:

It is important to keep all common areas clean and free of litter. Any dirty dishes or equipment should be cleaned and handled according to the manufacturer's instructions. Since shared facilities like refrigerators, incubators, or freezers are in common use, it's essential to label all solutions clearly. To avoid confusion, include initials or another unique identifier on the labels. Any unlabeled items found in these areas may be discarded. Additionally, always label the backs of plates with initials, the date, and any relevant experimental information to ensure proper identification. This will help maintain a clean and efficient lab environment for everyone.

Hazardous Chemicals and Material Safety Data Sheets

In a laboratory setting, many chemicals used can be hazardous, so it's crucial to understand the risks involved to ensure safety. All manufacturers of hazardous chemicals are legally required to provide users with detailed information on the hazards associated with their products through Material Safety Data Sheets (MSDS). These sheets serve as a guide for safe handling, storage, and emergency procedures. When encountering a new chemical in the laboratory, it is important to familiarise yourself with its properties and safety precautions. Record key details in your observation notebook, including the chemical's common and chemical name, molecular weight and required storage conditions (e.g., cold or room temperature). Always refer to the MSDS for information on hazards and safe handling. Some chemicals, such as phenol, which can cause severe burns, acrylamide, a potential neurotoxin, and ethidium bromide, a carcinogen, require extra caution. While these chemicals are safe when used properly, it's important to always wear gloves and avoid mouth-pipetting. If skin contact occurs, rinse the affected area thoroughly with water and inform the instructor immediately. Additionally, dispose of chemical waste in designated containers for hazardous

materials. By following these safety protocols and understanding the chemicals being used, a safe laboratory environment can be maintained.

Important Safety Protocols:

- Always read the MSDS before handling any hazardous chemicals.
- If an accident or spill occurs involving a hazardous chemical, **immediately inform the instructor** or lab supervisor.
- Follow the emergency procedures outlined in the MSDS to minimise harm.

Proper disposal of buffers and chemicals

Maintaining a safe and clean laboratory environment requires proper disposal and cleaning practices. Any unused solidified agar or agarose should never be discarded down the sink; instead, it must be disposed of in the appropriate trash, and the bottles should be rinsed thoroughly. If the media becomes contaminated, it should first be autoclaved before disposal. Biological waste must be placed in Biohazard containers for autoclaving before disposal. Organic reagents should always be handled in a fume hood and placed in labelled containers for proper disposal. Never dispose of these chemicals in the trash or sink. Dirty glassware should be rinsed well to remove traces of agar or other substances that cannot be cleaned in a dishwasher. Labels should be removed, and glassware should be placed in the dirty dish bin. Items like bottle caps, stir bars, and spatulas should not be put in the bin; instead, they should be washed with hot soapy water, rinsed well with hot water, and then rinsed three times with distilled water to ensure thorough cleanliness. These practices are essential for ensuring a safe and organised lab environment.

First-Aid, Treatment and Safety Equipment

Accidents might happen in spite of all precautions and care. It is essential for the students to know about immediate medical aid for a chemical accident and to learn about the safety devices to prevent them.

Fig.4.1: First Aid Box

Chemical poisoning and first-aid measures

Chemical poisoning may result from continuous contact or absorption through skin, inhalation of toxic vapour or swallowing it directly. Common symptoms of pesticide poisoning are headache, nausea, vomiting, tremors, convulsions and difficulty in respiration. A first-aid kit with necessary antidotes should be

available at the work site for each type of poisoning. Antidotes are always mentioned on the pesticide containers.

Treatment for simple chemical poisoning

I. **Swallowed poison:**If the poison has been swallowed, induce vomiting immediately. Mustard oil or table salt in a glass of warm water is good for this purpose. Touching the throat internally with a finger will also induce vomiting. The vomiting process should be continued till a clear liquid starts coming out of the stomach. If the poison is due to ingestion of mercury compounds, egg white and milk should be given first and then vomiting should be induced. And then soothing substances like raw egg white (mixed with water), butter, or cream milk must be given.

II. **Skin contamination:** Contaminated clothes may be removed at once. Contaminated skin should be washed with soap and water and also flushed with plenty of water to reduce the extent of injury.

III. **Eye poisoning**: The Eyes of the victim may be washed with plenty of water, keeping the eyelids open. A quick, decisive action is desirable as delay of a few seconds may greatly increase the extent of the injury. Refer to an eye doctor immediately.

IV. **Inhaled poison**: The victim of inhaled poison must be immediately exposed to an open area with fresh air. Keep the patient quiet as far as possible. Provide a blanket to avoid chilling. If breathing stops, the artificial respiration technique through the mouth may be used.

Safety and protective equipment

Protective and safety equipment minimise the chances of a major accident. The protective and safety equipment essentially includes a gas mask, hand gloves, shoes, eye shields, headgear, protective clothing, respiratory devices, etc.

I. Gas mask: It is a device to protect the eyes and respiratory tract from toxic gases and aerosols. It gives clean air to the operator by removing contamination from the ambient air by using a filter or a bed of absorbent material.

II. Hand gloves: It is used for protecting hands**.** Always use rubberised waterproof gloves instead of cotton leather or any fluid-absorbing material.

Fig. 4.2: Hand-gloves and mask for the head

III. **Shoes:** While working in the laboratory, wear rubber or any synthetic waterproof shoes instead of leather or canvas shoes.

IV. **Eye shields**: These must be worn to prevent eye poisoning.

V. **Protective clothing:** Wear an apron as protective wear to protect both the clothes you have worn. This also protects your body and skin.

Fig.4.3: Protective clothing

Health and safety awareness in the workplace

- Encourage seniors to keep an eye on those working in the workplace.
- Use charts and visuals to demonstrate commitment to health and safety.
- Encourage safe work practices while discouraging unsafe work practices.
- Even at the cost of repetition, communicate that safety is of prime importance while at work.
- Respond and act promptly to all health and safety concerns.

- Set an example in the use of all preventive and protective materials and practices.
- Keep young trainees away from the operational area, or supervise them personally to ensure that they do not come close to equipment which they are not yet trained to use.

Amenities and environment

- Train all workers rotationally in the use of first aid equipment and provide first-aid kits at accessible points.
- Insist on first-aid training for all the laboratory workers.
- There should be free access to washrooms and toilet facilities with running water or stored clean water.
- There should be free access to potable, clean, and cool drinking water.
- Don't keep flammable materials in large quantities or easily approachable or accessible areas prone to fire hazard

Emergency response

- Train a task force for emergency response action for the workplace (for example, snakebite, fire, confined space entry, heat stress, or chemical spill).
- Keep the safety awareness level of workers high at all times.
- Maintain emergency response equipment.

General Laboratory Safety instructions:

Do's:

1. Always wear appropriate Personal Protective Equipment (PPE) such as lab coats, gloves, safety goggles, and closed-toe shoes.
2. Familiarise yourself with the location and proper usage of safety equipment, including fire extinguishers, emergency showers, eyewash stations, and first aid kits.

3. Follow established protocols for handling, storing, and disposing of hazardous materials, including chemicals, biological samples, and radioactive substances.

4. Keep the work area clean, tidy, and free from clutter to prevent accidents and ensure easy access to emergency equipment.

5. Clearly label all chemicals, reagents, and samples with their contents, date, and any relevant hazard warnings.

6. Conduct experiments and procedures following approved protocols and under appropriate supervision.

7. Report any accidents, spills, or incidents to your supervisor or the designated personnel immediately, no matter how minor they may seem.

8. Maintain clear communication with your colleagues and inform them of any potential hazards or changes in experimental conditions.

9. Stay up-to-date on safety training and attend refresher courses regularly.

10. Stay vigilant and be mindful of your surroundings at all times.

Don'ts:

1. Don't neglect to wear or improperly wear PPE when working in the laboratory.

2. Don't eat, drink, or store food in the laboratory areas.

3. Don't pipette by mouth or directly taste or smell chemicals.

4. Don't perform unauthorised experiments or procedures without proper training and supervision.

5. Don't use damaged or malfunctioning equipment. Report any issues promptly.

6. Don't store incompatible chemicals together, and avoid mixing chemicals unless instructed to do so by an authorised individual.

7. Don't block emergency exits or pathways with equipment or supplies.

8. Don't engage in horseplay or distract others during experiments or procedures.

9. Don't remove or tamper with safety devices or equipment.

1. Don't rush or take shortcuts that compromise safety measures or experimental integrity.

Practical Exercises

Activity 1

Identification of the components of a first aid kit.

Materials required: First aid kit, bandages, antiseptic wipes, gauze pads, adhesive tape, scissors, tweezers, cotton balls, thermometer, gloves, pain relievers, antiseptic cream, burn ointment, etc.

Procedure:

1. Open the first aid kit and look at the items inside.
2. Identify the different items like bandages, gauze pads, and scissors.
3. Learn how to use each item:
    - Bandages and gauze for covering wounds.
    - Scissors for cutting bandages or tape.
    - Tweezers for removing splinters or dirt.
    - Antiseptic wipes and cream for cleaning wounds.
4. Understand when to use items like pain relievers or burn ointment.

Activity 2

Identification of the components of a first aid kit.

Materials required: Safety goggles, gloves, ear protection (earmuffs or earplugs), face masks, hard hats, aprons, safety boots, reflective vests, first aid kit.

Procedure:

1. Identify safety devices and write down the purpose of each device.
2. Demonstrate how to wear the device properly.
3. Discuss with experts that when and why each device should be used.

Check Your Progress

A. Multiple Choice Questions

1. Common symptoms of pesticide poisoning are:
   a) Headache
   b) vomiting and nausea
   c) difficulty in respiration
   d) All of these
2. To prevent hazards at working place, availability of following materials should be ensured:
   a) SDS
   b) First-aid kits
   c) Protective clothing
   d) All of these
3. Emergency services comprise .
   a) Ambulance
   b) Fire brigade
   c) Both (a) and (b)
   d) None of these
4. Potential dangerous creatures around house and office buildings include .
   a) lizards
   b) snakes
   c) spiders and scorpions
   d) All of these
5. What safety measures are required during the application of pesticides to the crop?
   a) Mixing the correct quantity of pesticide and clean water, and spraying during evening time
   b) Use of any type of nozzle and spray mixture
   c) Spraying of insecticides with flat nozzle against the direction of wind
   d) Spraying at any time during the day

B. Fill in the Blank

1. To induce vomiting,_____can be used.
2. Contaminated skin must be_____.
3. To protect eyes and respiratory tract from toxic gases,_____ is used.
4. Hand gloves made up of_____are used to handle chemicals.
5. For inhaled poison, first-aid can be_____.

Match the Column A & B

	Column A	Column B
1.	Eye	a. Rubber
2.	Shoe	b. shield
3.	Protective Clothes	c. Apron

C. Subjective Questions

1. What are the first-aid treatment measures for chemical poisoning?
2. What protective devices are meant for protection in the agricultural field?
3. Define agro-chemicals.
4. Discuss the various harmful effects of agro-chemicals

Session 2: Safe Use of Lab Equipment

Laboratory operations today rely heavily on various lab equipment. The use of this equipment requires careful handling with all necessary safety precautions. Accidents related to laboratory equipment are caused due to the following reasons:

- Lack of adequate or proper training for workers.
- Poor maintenance of tools and equipment.
- Using equipment that is not suitable for the task at hand.
- Failure to follow proper safety protocols during experiments.
- Missing or defective safety features on equipment, increasing the risk of accidents.
- Unsafe methods for resolving equipment malfunctions or blockages during experiments.

Checking Lab Equipment Before Use

Before beginning any work with lab equipment, ensure it is in proper working condition and safe to use. While the requirements may vary depending on the equipment, basic checks should always be performed:

- Consult the equipment's manual for pre-operational instructions and follow them carefully.
- Pay special attention to components like switches, moving parts, electrical cords, and any attachments that need to be checked before use.
- Ensure that guards, covers, and protective barriers are securely in place and won't come loose.
- Immediately repair or replace any defective or damaged parts. Emergency stop mechanisms and other safety devices should function correctly, such as power switches or circuit breakers.
- When attaching or connecting components, ensure they fit securely and are of the correct size or specification. Avoid makeshift solutions or improper fittings.
- Equipment with moving parts should have clear and accessible safety mechanisms in place, and proper warning indicators should be visible before use.

Safety precautions to follow during the use of lab equipment:

- Before using any lab equipment, read and understand the user manual and safety warnings.
- Always wear Personal Protective Equipment (PPE) such as lab coats, gloves, and safety goggles while handling lab equipment.
- Inspect equipment for any damage or malfunction, such as frayed wires or cracks, before use.
- Use the equipment only for its intended purpose, avoiding misuse that could cause accidents.
- Operate equipment that emits fumes, vapours, or dust in a well-ventilated area or under a fume hood.
- Ensure all electrical equipment is properly grounded to prevent electrical shocks or short circuits.
- Follow the recommended calibration and maintenance schedules to keep equipment functioning safely and accurately.
- Never leave running equipment unattended, especially when using heating or high-voltage devices.
- Keep the area around the equipment clear of clutter and flammable materials to avoid accidents.
- After use, turn off and, if necessary, unplug the equipment to prevent electrical hazards or fires.
- Ensure all cords, plugs, and connections are in good condition and not damaged to avoid electrical hazards.
- Use appropriate tools, such as insulated handles, when dealing with high temperatures or electricity to protect yourself.
- Always operate centrifuges with balanced loads to prevent mechanical failure or damage.
- Keep liquids away from electrical equipment to prevent spills that could lead to short circuits or electrocution.
- Be familiar with the emergency shutdown procedures for equipment in case of malfunction or fire.
- Store equipment and tools properly after use, ensuring that they are in designated places and do not create obstructions.

Daily or Periodic Mandatory Inspection for the Use of Lab Equipment

- Check connections to power sources, water, or gas supplies, depending on the equipment.

- Inspect hoses, lines, and connectors for signs of wear, cracks, or leaks.
- Once equipment is running, check all control settings (like power switches or speed dials) to ensure everything is functioning properly.
- Inspect metal parts or frames for cracks or corrosion that could cause equipment failure.
- Maintain a safe distance from hazardous equipment when it's in operation, and avoid unnecessary physical proximity to moving parts.
- Ensure electrical wires or cables are not in contact with any heat sources or flammable chemicals.
- Report any unsafe observations to supervisors to take preventive measures.

Protective Measures during Lab Equipment Operation

Wearing Personal Protective Equipment (PPE) is crucial for laboratory safety. All personnel must wear appropriate PPE to protect against accidents and hazards. Ensure the protective clothing or gear fits properly and provides complete coverage, without loose ends or flammable materials. Features of protective dress and equipment include:

- Proper fit, cleanliness, and maintenance.
- Safe storage to prevent damage, contamination, or wear.
- No rough or sharp edges on protective gear.
- Full body coverage through lab coats, aprons, gloves, and safety shoes.
- Use ear protection if noise levels are high, such as when operating equipment like centrifuges.
- Always wear goggles, face shields, or safety glasses for eye protection.
- Ensure that PPE is specific to the part of the body being protected and is readily available.
- Keep PPE clean, functional, and sanitised regularly to maintain effectiveness.

Practical Exercises

Activity 1

Demonstration of general inspections for use of lab equipment.

Materials required: Lab equipment, gloves, cleaning supplies, inspection checklist, pen, pencil, notebook, etc.

Procedure:

1. Check lab equipment's for cleanliness and ensure there are no cracks or damage.
2. Clean equipment regularly exercising care.
3. Ensure that all parts of equipment, are functioning properly.
4. Regularly inspect the equipment.

Activity 2

Precautions taken during the use of lab equipment.

Materials required: Lab equipment, gloves, safety goggles, lab coat, fire extinguisher, first aid kit.

Procedure:

- Wear safety equipment such as gloves, safety goggles and a lab coat before handling any equipment.
- Ensure that all equipment is properly set up and checked for any damage before use.
- Handle glassware carefully to avoid breaking, and always use proper techniques while using pipettes or microscopes.
- When using a Bunsen burner, check for gas leakages and always turn it off when not in use.
- Keep safety equipment, such as fire extinguishers and first aid kits in proper place for use in emergencies.

Check Your Progress

A. Multiple Choice Questions

1. What is necessary to check machinery before start?
   a) Farm operations
   b) Fill the fuel
   c) check the tires
   d) check the lights
2. What type of care is required to avoid any machinery accident?
   a) Using a machine that is unsuitable for the task
   b) Using unsafe methods for operations
   c) Guards and other safety devices missing or defective
   d) Using safe operating procedures

3. Which of the following safety precautions are necessary while refueling of tractor or other machinery?
   a) Engine in running condition
   b) Engine in off position
   c) Engine in off and no open flame nearby
   d) All of these

B. Fill in the Blank

1. During harvesting ensure that the operators should wear _____ and secure their _____to avoid entanglement.
2. Need to protect not to allow anyone to _____ onto the machine while it is in motion.
3. Operators must wear _____clothing.

Subjective questions

1. Enlist the general inspections of the machinery before use.
2. Describe the health and safety during combine harvesting.
3. Describe the use of protective clothing during machinery operations.

Glossary

Abiotic Stress: Non-living environmental factors (e.g., temperature, light intensity, humidity) that can negatively affect plant growth and development.

Adventitious Shoots: Shoots that arise from non-meristematic tissue such as leaves or roots, not from pre-existing buds.

Antidote: A substance that counteracts the effects of poison.

Antitranspirant: Chemical substances applied to plants to reduce water loss through transpiration during stressful conditions such as acclimatization.

Artificial Respiration: A first aid procedure to assist or stimulate breathing by manually forcing air into the lungs.

Autotrophic: The ability of plants to synthesize their own food through photosynthesis using light energy, carbon dioxide, and water.

Aseptic Conditions: A sterile environment free from living microorganisms, necessary to prevent contamination during in vitro culture.

Auxins: A class of plant hormones that regulate root formation, cell elongation, and differentiation, commonly used in tissue culture to promote root formation.

Biohazard Container: A container specifically designed to safely hold biological waste materials that may be infectious or hazardous.

Biotic Stress: Living organisms (e.g., microbes, pests, pathogens) that can cause harm or stress to plants.

Bioreactor: A controlled system for large-scale tissue culture propagation using liquid media.

Calibration: The process of adjusting and standardizing equipment to ensure accurate and reliable measurements.

Callogenesis: The induction and growth of callus tissue from explants in vitro.

Carcinogen: A substance or agent that can cause cancer in living tissue.

Chemical Compatibility: The ability of chemicals to be stored or handled together without adverse reaction.

Chemosterilant: A chemical agent used to eliminate microbial contamination on explants without damaging plant tissues.

Contaminant: Any unwanted microorganism (such as bacteria or fungi) that invades cultures and potentially ruins experiments.

Cryopreservation: A technique of preserving plant genetic material at ultra-low temperatures for long-term storage.

Culture Medium: A nutrient-rich artificial substance (solid or liquid) used to support growth and development of plant tissues in vitro.

Culture Vessel: Containers such as test tubes, jars, or flasks where explants are grown under sterile conditions.

Decontamination: The process of removing, cleansing, or neutralizing hazardous substances from equipment, surfaces, or personnel.

Disinfectant: Chemical agents (e.g., ethanol, sodium hypochlorite) used to reduce or eliminate pathogens from surfaces and tools.

Ergonomics: The study of designing equipment and workspaces to fit the user's needs and reduce strain or injury.

Explant: A small piece of plant tissue (e.g., leaf, stem, meristem) used to initiate a tissue culture.

Ex vitro: Plants grown outside the laboratory environment, typically in soil or natural conditions, as opposed to in vitro (lab culture) conditions.

Fumigation: Use of gases (like formaldehyde) to disinfect closed areas such as culture rooms.

Fume Hood: A ventilated enclosure in a laboratory used to limit exposure to hazardous or toxic fumes, vapors, or dust.

Genetic Fidelity: The degree to which tissue culture plants maintain the genetic characteristics of the original plant.

Gloves and Lab Coats: Personal protective equipment (PPE) worn to minimize contamination risks and protect personnel.

Hardening (Hardening Off): The process of gradually acclimatizing tissue culture plants by exposing them to less humid and more natural conditions before transplantation.

HEPA Filters: Filters in laminar flow hoods that remove particles and microbes from the air to maintain cleanliness.

Inhalation Hazard: Risk posed by breathing in harmful substances.

***In vitro*:** Refers to processes performed in a controlled environment outside a living organism, such as in culture vessels.

Labelling: The practice of clearly marking containers with contents and hazard information.

Meristem: A region of actively dividing cells in plants responsible for growth, often used for virus-free cultures.

Microflora: The community of microscopic organisms such as bacteria and fungi living in soil or other environments.

Micro-shoot: A small shoot generated in vitro during micropropagation.

Murashige and Skoog Medium (MS Medium): A widely used nutrient medium formulation for plant tissue culture that provides essential nutrients for growth.

Nodal Segment: A stem segment containing a node, commonly used as explants for axillary shoot proliferation.

Organogenesis: The process of forming organs like shoots and roots from callus or explants during tissue culture.

Pathogen: Disease-causing organisms that may be introduced into cultures or pose risks to workers.

Phenolic Browning: A common problem in tissue culture where plant tissues produce brown pigments that inhibit growth.

Photoperiod: The duration of light exposure in a 24-hour period, influencing plant growth and development.

Photosynthetic Efficiency: The effectiveness with which plants convert light energy into chemical energy during photosynthesis.

Plant Growth Regulators (PGRs): Natural or synthetic substances that influence plant growth and development, including auxins and cytokinins.

Personal Protective Equipment (PPE): Clothing and gear designed to protect the wearer from hazards (e.g., gloves, goggles, lab coats).

Reactive Chemicals: Chemicals that can undergo violent chemical change when exposed to certain conditions.

Relative Humidity: The amount of moisture in the air expressed as a percentage of the maximum amount the air can hold at a given temperature.

Risk Assessment: The systematic process of evaluating potential risks that may be involved in a projected activity or undertaking.

Root System Architecture: The spatial configuration of a plant's root system including depth, branching, and density which affects nutrient and water uptake.

Somaclonal Variation: Genetic variation observed among plants regenerated from tissue culture, sometimes used for developing new traits.

Sterilization: The process of eliminating all living microorganisms from instruments, culture media, or explants to prevent contamination in tissue culture.

Totipotency: The capacity of a single plant cell to develop into a complete plant under suitable conditions.

Transpiration: The process by which water vapor is lost from plant leaves to the atmosphere, important in plant water regulation.

Answer Keys

Unit 1: Preparation of Mother Plant and Explant for Plant Tissue Culture

Session 1: Selection and Pre-Treatment of Mother Plant

A. Fill in the blanks

1. Mother plant
2. Explant
3. NaCl
4. Sucrose

B. Multiple Choice Questions

1-B, 2-c, 3- b, 4-d, 5 -a

Session 2: Different Plant Tissue Culture Techniques

A. Fill in the blank s

1- Clonal propagation
2- Cytokinin
3- Organogenesis
4- Callus

B. Multiple Choice Questions

1-b, 2-d, 3-a, 4-b, 5-c

Unit 2: Inoculating The Explant and Its Multiplication

Session 1: Excising of explant from Mother Plant

A. Fill in the Blanks

1- Contamination
2- ethanol
3- Nutrient
4- laminar airflow
5- Sterile distilled water

B. Multiple Choice Questions

1-b,2-c, 3-a, 4-b, 5-c

Session 2: Preparing and Inoculating an Explant into The Culture Medium

A. Fill in the Blanks

1- Stage 0
2- Meristem
3- IBA
4- Hardening

B. Multiple Choice Questions

1-B, 2-C,3-B, 4-A, 5-B

C. Match the following

1 -C, 2 -B, 3 -D, 4 -A

Unit 3: Acclimatizing The Tissue-Cultured Plants

Session 1: Process of Acclimatization (Hardening)

A. Fill in the Blanks

1- biotic, abiotic
2- nutrient solution
3- Hoagland
4- shock

B. Multiple Choice Questions

1-B, 2-C,3- A,4-C, 5-D

Session 2: Types of Crops Suitable for Tissue Culture and Resource Optimisation

A. Fill in the Blanks

1- banana
2- disease
3- Glass bottles

B. Multiple Choice Questions

1-B, 2-C-,3-C, 4-B

Unit 4: Maintain Health and Safety At Work Place

Session 1: First-Aid, Treatment and Safety Equipment

A. Fill in the Blanks:

1) Table Salt and Mustard Oil
2) Wash with soap
3) Gas Mask
4) Rubber
5) Artificial Respiration

B. Multiple Choice Questions

1.- D, 2.- D, 3.- C, 4.- D, 5.- A,

C. Match the Column

1.-B, 2.- A, 3.- C

Session 2: Safe Use of Lab Equipment

A. Fill in the Blanks:

2. Climb
3. Protective

B. Multiple Choice Questions:

1.-C, 2.- D

List of Credits

DAAH, PSSCIVE, Bhopal

- *Fig. 1.1*
- *Fig. 1.2*
- *Fig. 1.4*
- *Fig. 1.7*
- *Fig. 1.9*
- *Fig. 2.1*
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- *Fig. 3.2*

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- *Fig. 1.3*
- *Fig. 1.5:*
- *Fig. 1.6*
- *Fig. 1.8*

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- Fig.4.1: Courtesy: https://shorturl.at/3OIx9
- Fig. 4.2: Courtesy: https://goo.gl/ZvGFsn
- Fig.4.3: Courtesy: https://goo.gl/mzBA2Q