Draft Study Material

PLANT TISSUE CULTURE TECHNICIAN

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

SECTOR: AGRICULTURE

Grades 11

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NCERT

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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: 20 June 2024

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Table of Contents

Module 1

Introduction to Plant Tissue Culture

Module Overview

The Plant Tissue Culture technique involves the multiplication of plant cells or organs under aseptic conditions, supplemented with a nutritionally defined medium, and controlled conditions of light, temperature, and humidity (*in vitro*). Plant tissue culture includes the regeneration of complete plants from explants such as shoots, leaves, roots, anthers, embryos, single cells, and protoplasts. It is a modern technique for research and commercial applications, based on the principle of regeneration of a plant cell known as *totipotency*.

Totipotency is the ability of a vegetative cell to divide and differentiate into any type of specialized cell or regenerate into a whole plant. Morgan (1901) coined the term *"Totipotency"* to denote the capacity of a cell to develop into an organism by regeneration. This unique and important property of plant cells known as *Totipotency* is mainly applicable in tissue culture.

Explants are used to initiate cell cultures that ultimately develop into complete plantlets (a small, young plant through a process known as micro propagation. Micro propagation derives its name from miniature shoots or plantlets produced by this method. It is also known as *in vitro* micro propagation, the term "*in vitro*" originates from the Latin word " vitreum" meaning glass. An explant of a desired plant is cultured *in vitro* on a synthetic medium that promotes the rapid multiplication of cells. The new plants are then removed from the culture and transferred to a standard potting medium (soil + vermiculites).

This technique is particularly important for plants that are difficult to propagate traditionally. In 1960, George Morel demonstrated the potential of clonal micro propagation by producing several million orchids (Cymbidium) plantlets in one year from a single shoot tip explant. This method allows for the commercial production of a large number of plantlets.

Learning Outcomes

After completing this module, you will be able to:

1. Understand the basic techniques of plant tissue culture and their role in plant propagation.

2. Identify the potential of plant tissue culture in improving crop production and its future in India.

Module Structure

- Session 1: Plant Tissue Culture Techniques and Its Importance
- Session 2: Scope and Prospect of Plant Tissue Culture in India

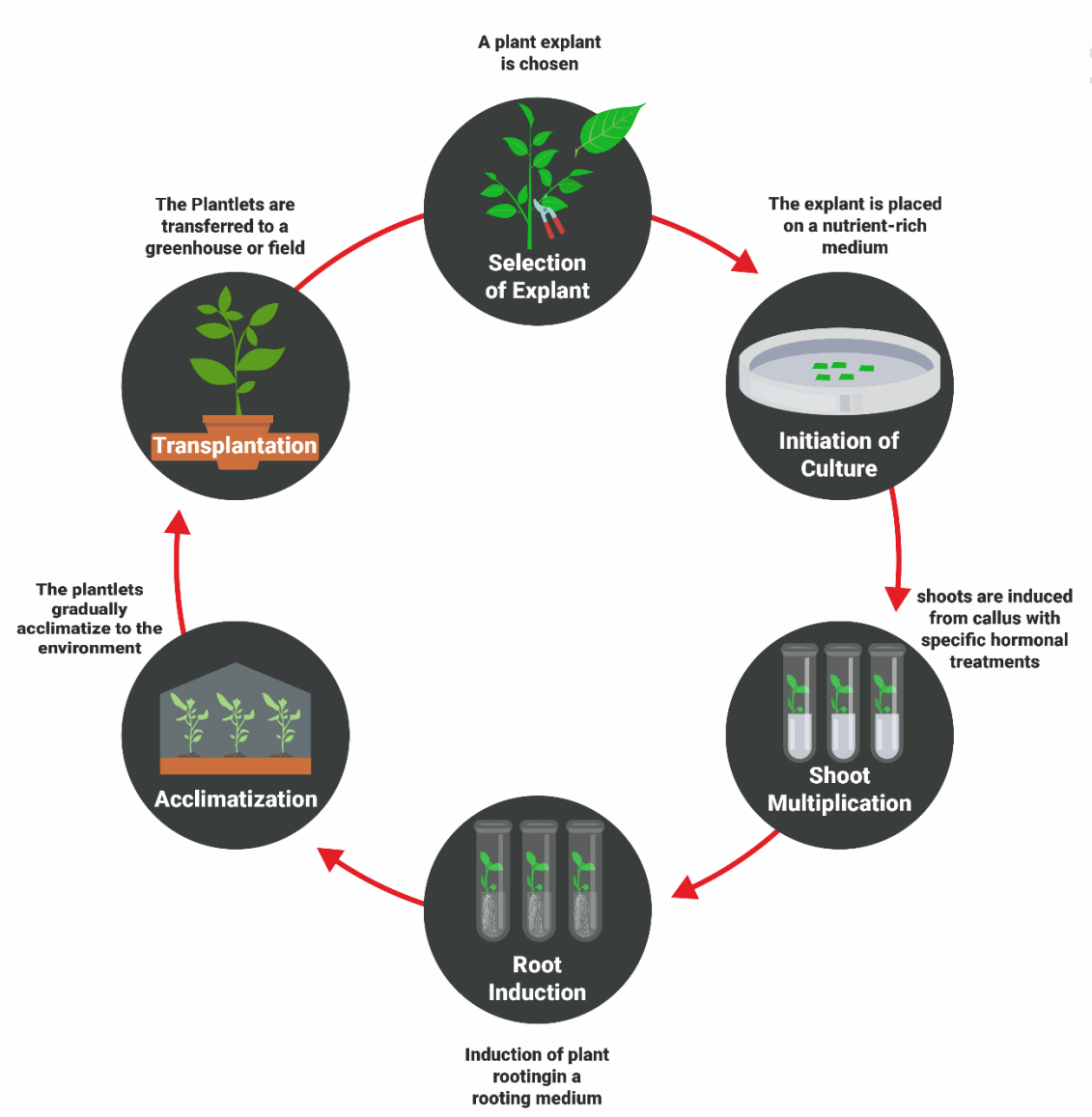

Fig: 1:1 Process of Micro propagation

Session 1: Plant Tissue Culture Techniques and its Importance

Plant tissue culture is an important technique in the field of plant biotechnology. Plant tissue culture is a practice used to propagate plants under sterile conditions, used to produce plants that are genetically identical to their parents. Tissue culture techniques offer various advantages over traditional methods of propagation including the production of true-to-type

plants even in the absence of seeds or low germinating seeds, the production of genetically modified and disease-free plants. This method is widely used as an alternative method of vegetative propagation for the mass multiplication of plants. The newly regenerated plants are then hardened under controlled conditions before being planted in soil and grown in the open field, where they continue to develop as normal plants. Another important application of the method is the recovery of healthy plants from diseased plants (used for the independence of viral-free plants). Although the plant is infected with a virus, the meristem (apical and axillary) is free of virus. By removing the meristem from a plant and cultivating it *in vitro* to produce virus-free plants. Researchers have successfully cultured the meristems of various plants such as bananas, sugarcane, and potatoes. They have also isolated single cells from plants, and after digesting the cell walls, obtained naked protoplasts surrounded by plasma membranes. Protoplasts from two different plant varieties, each possessing desirable traits, can be fused to create hybrid protoplasts. These hybrid protoplasts can then be cultivated to develop into new plants.

Basics and Principles of Plant Tissue Culture

Tissue culture techniques are based on the Concept of *Totipotency*; which refers to the ability of plant cells to develop into a complete plantlet. *Totipotency* is the potential of cells which allows them to grow and develop into a multicellular organism.

In 1902, German botanist Gottlieb Haberlandt introduced the concept of *Totipotency* but failed to prove it experimentally when he tried to grow isolated cells of *Tradescantia* plants under *in vitro* conditions. Although the cells enlarged, they did not divide. But with this experiment, he laid the foundation of Plant Tissue Culture and is aptly called the 'Father of Plant Tissue Culture'.

Did you know?

- German botanist Gottlieb Haberlandt is known as the Father of Plant Tissue Culture.

During the 1930s, the discovery of auxin as a Plant Growth Regulator (PGR) and the importance of vitamins in culture media laid the foundation of plant tissue culture. In 1934, Gautheret cultured cambial cells of Salix plants in the presence of a natural auxin called IAA (Indole-3-acetic acid) and Vitamin B.

Tissue culture medium contains all the essential nutrients required for the growth and development of plants in aseptic conditions. The culture medium primarily consists of macronutrients (Nitrogen, Phosphorus, Potassium), micronutrients (Calcium, Sulfur, Magnesium, Iron, Zinc etc.), vitamins,

various organic compounds, plant growth regulators, a carbon source, and, solidifying agents. Growth regulators are crucial in the development of plant cells and tissues in the culture medium. Among these, auxins, cytokinins, and gibberellins are the most commonly used Plant Growth Regulators (PGRs) in tissue culture and micropropagation.

Advantages of Plant Tissue Culture

- Plants produced through tissue culture are true-to-type.
- Tissue culture can quickly produce mature plants.
- Plants that cannot multiply by seed can easily be propagated/multiplied by tissue culture techniques.
- Plant tissue culture techniques are helping to produce disease-free, viral-free plants.
- In tissue culture, plants multiply in controlled environments, allowing for year-round multiplication.
- This method allows the multiplication of a large number of plants in a short period.

Disadvantages of Plant Tissue Culture

While tissue culture presents various advantages, it also has certain limitations:

- Tissue culture is labor-intensive operations and requires skilled personnel.
- It is an expensive method of plant propagation as it necessitates well-equipped laboratories and trained personnel.
- Tissue-cultured plants can be more sensitive to environmental conditions since they have been grown in controlled laboratory settings.
- Contamination poses a significant challenge in tissue culture labs.
- Tissue-cultured plants are costlier compared to conventionally propagated plants.

Types of Plant Tissue Culture

1. Callus cultures

Callus is an undifferentiated mass of parenchymatous cells which are disorganized and often produced upon wounding. The parts of leaves or stems are inoculated onto the medium under *in vitro* conditions. The tissues are first dedifferentiated into undifferentiated unorganized mass which is called callus. Large-scale callus culture can be produced in flasks and bottles as shown in figure 1.1. Each cell of the callus is capable of giving rise to a plantlet on the principle of *totipotency*. Manipulation of culture medium and PGR cause callus cells to re-differentiate into shoots/roots (organogenesis) or

embryos (somatic embryogenesis). The unique feature of callus is that abnormal growth has the potential to develop normal shoots, roots, and embryoids, ultimately developing a plant.

Fig: 1.2: Callus culture in jars showing explants forming undifferentiated cells on nutrient media

2. Organ culture

The culture of different plant organs is referred to as organ culture. These include Shoot culture, Root culture, Anther and Pollen culture, Embryo culture, and Ovary culture.

i. **Shoot culture** - The shoots (which contain the shoot apical meristem) can be cultured *in vitro*, producing clumps of shoots from either axillary or adventitious buds. This method can be used for clonal propagation.

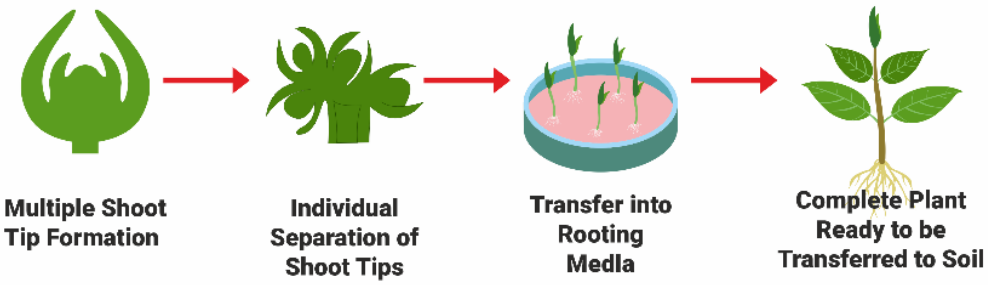

Fig: 1.3: Shoot culture showing shoot proliferation from explants on nutrient media

ii. **Root culture:** Root cultures can be established *in vitro* from explants of the root tip of either primary or lateral roots and can be cultured on fairly simple media. The growth of roots *in vitro* is potentially unlimited, as roots are indeterminate organs. Although the establishment of root cultures was one of the first achievements of modern plant tissue culture, they are not widely used in plant transformation studies.

iii. **Anther culture:** Culturing anther on a suitable media to regenerate into haploid plants is called anther culture. The first time, haploid plants were discovered in *Datura stramonium* by A.D. Bergner in 1921.

iv. **Ovary Culture:** Ovary culture is also used to produce haploid plants. The unpollinated ovaries are used for this purpose. It is possible to trigger the female gametophyte of angiosperm to develop into a sporophyte. The plants produced by this culture are called gynogenic haploid.

v. **Embryo Culture:** Embryos can be used as explants to generate callus cultures or somatic embryos. Both immature and mature embryos can be used as explants. Immature, embryo-derived embryogenic callus is the most popular method of monocot plant regeneration.

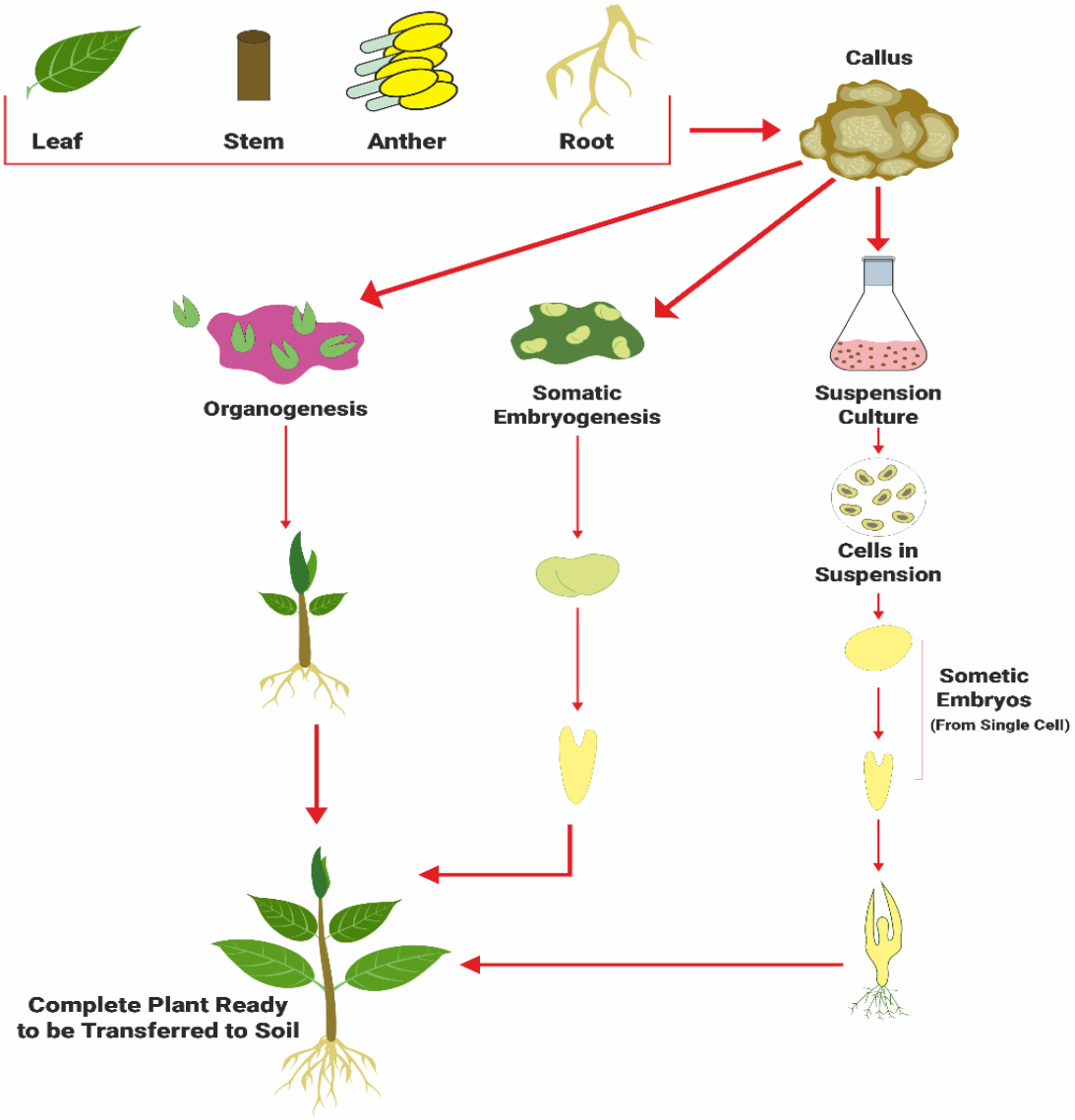

Fig: 1.4: different types of organ culture

3. Cell cultures

A single cell has the ability to regenerate into a complete plantlet in a liquid nutrient medium under *in vitro* conditions, this process is known as cell suspension culture. Single cells can be isolated from the callus or any other part of the plant and transferred via a spatula onto a liquid culture media. The medium needs to be agitated for uniform availability of nutrients and air. The cell suspension culture needs to be subculture onto fresh medium or fresh liquid medium could be added periodically. Usually, the medium contains the auxin 2,4-D. Suspension cultures must be constantly agitated at 100-250 rpm (revolutions per minute). Suspension cultures grow much faster than callus cultures. It can be used for scaling up and automation *via* a Bioreactor.

4. Protoplast Culture

A protoplast is the living part of a plant cell, consisting of the cytoplasm and nucleus without a cell wall. Protoplasts can be isolated from whole plant organs or tissue cultures. Protoplasts are fragile and get easily damaged and therefore must be cultured carefully. The liquid medium must be shallow enough to allow aeration without agitation. Protoplasts can be plated out on solid medium and callus-produced. Whole plants can be regenerated by organogenesis or somatic embryogenesis from this callus. Protoplasts are ideal targets for transformation by a variety of means.

5. Somatic Embryogenesis

Callus can be induced to form a group of somatic embryos, which resemble the seed embryos of intact plants but lack a seed coat. These somatic embryos can develop into seedlings through bipolar germination. This process is known as somatic embryogenesis (Figure 1.7). Somatic embryos were first reported by F. C. Steward (1958) and Reinert (1959) from carrot cell suspension culture.

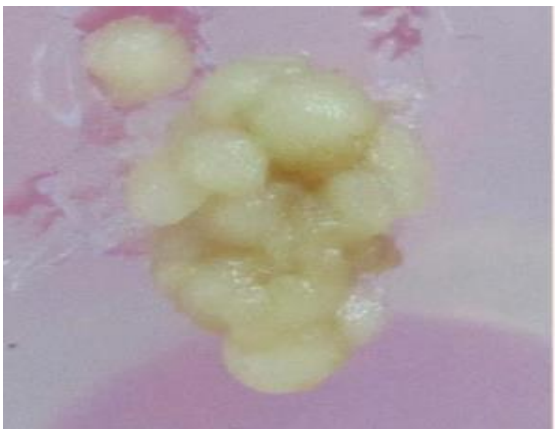

Fig: 1.5: Somatic Embryogenesis

Automation in Plant Tissue Culture

Plant Tissue Culture is an experimentally proven technology for the *in vitro* production of many plantlets. The technology is expensive and labor-intensive and requires complicated and controlled aseptic conditions wherein human error causes tremendous damage and loss of revenue. Automation at various stages of tissue culture reduces the input cost. The automation is being done at different commercial levels, such as;

(i) Plant Growth Chambers, where all environmental parameters (light intensity, light duration, humidity, carbon dioxide concentration, temperature etc.) can be automatically set with the help of timers and monitored at all times.

(ii) The preparation of Culture media is also automated at the commercial level for uniformity and accurate measurement of nutrients and pH. Dispensing of the medium into culture vessels is also automated to enhance time management, and accuracy, and maintain aseptic conditions.

(iii) Washing of glass wares and maintenance of aseptic conditions are all automated in commercial labs.

(iv) Environment and parameter control in the Culture room/Greenhouse via light and temperature controller.

(v) Temporary Immersion Systems (TIS) bioreactors are now utilized for micropropagation purposes. Bioreactors are also employed for the production of secondary metabolites from plant cultures. These systems offer controlled environments and optimal nutrient delivery, leading to enhanced productivity and reproducibility.

Economic Status of Tissue Culture in India

In India, agriculture plays a vital role, employing approximately 54% of the workforce. Its growth has notably contributed to poverty reduction, regional economic balance, and food security enhancement. Given the projected population surge to 1.7 billion by 2050, ensuring sustainable food production and distribution is a paramount task for researchers and policymakers alike.

Biotechnological approaches, particularly tissue culture, are increasingly becoming relevant to enhance both the quality and economic sustainability of crop production. These techniques play crucial roles in the rapid development of disease-resistant crops and elite plant varieties compared to traditional breeding methods. Tissue culture facilitates the propagation of plants that are difficult to propagate conventionally and reduces the dormancy period of crops, facilitating faster multiplication of plants than

traditional methods. Government institutions like the Department of Agricultural Research and Education (DARE) and Department of Biotechnology (DBT), under the Indian Council of Agriculture Research (ICAR) spearhead the adoption of plant biotechnology and tissue culture for crop improvement, with various institutes focusing on enhancing specific crops such as fruit crops, spices, pulses, oilseeds, and vegetables, etc.

The Government of India has established the National Certification System for Tissue Cultured Raised Plants (NCS-TCP), for the maintenance of quality standards and elimination of the distribution of inferior plantlets. Government schemes and incentives, including subsidies on investments also support the growth of plant tissue culture units. India's plant tissue culture industry is primed for growth due to factors like cost-effective skilled labour, scientific expertise, rich plant biodiversity, and a favourable tropical climate. With concerted efforts from stakeholders such as government regulatory bodies, research institutions, and industry; India's plant tissue culture sector has the potential to emerge as a significant player in global agriculture markets, contributing substantially to economic growth and food security.

ACTIVITIES

Visit a nearby Plant Tissue Culture Lab and observe its components.

Material Required

- Pen
- Pencil
- Notebook

Procedure

1. Visit the nearby Plant Tissue Culture Lab.
2. Observe and note down the following:
    a. Enlist different components of the laboratory.
    b. Identify and enlist commonly propagated plants through plant tissue culture.
    c. Enlist different techniques used in plant tissue culture.
    d. Discuss with the lab manager about functioning of tissue culture lab.
    e. Note down any other observations during the visit.
    f. If you have any queries discuss with the lab manager.

CHECK YOUR PROGRESS

A. Fill in the blanks

1. Plant tissue culture involves the cultivation of plant cells or organs under _____ conditions.
2. Micro-propagation is also known as ____Vitro clonal propagation.
3. _____ is the ability of a plant cell to develop into a complete plantlet.
4. Callus is an undifferentiated mass of _____ cells.

B. Multiple choice questions

1. What is the main purpose of plant tissue culture?
   a. Genetic engineering
   b. Mass multiplication of plants
   c. Soil fertility improvement
   d. Water conservation
2. Which plant growth regulators are commonly used in tissue culture?
   a. Vitamins
   b. Carbon source
   c. Auxins, cytokinins, gibberellins
   d. Micronutrients
3. What is the purpose of automation in plant tissue culture?
   a) Reduce cost and labor
   b) Increase contamination
   c) Slow down the process
   d) Decrease accuracy
4. Which scientist is referred to as "Father of Plant Tissue Culture"?
   a) George Morel
   b) Gottlieb Haberlandt
   c) Gautheret
   d) Murashige

C. Subjective questions

1. Explain the basic principles of plant tissue culture.
2. Explain different types of plant tissue culture techniques and their applications.
3. Discuss the advantages and limitations of plant tissue culture for plant propagation.

Session 2: Scope and Prospect of Plant Tissue Culture in India

Plant tissue culture is an emerging technology that impacts agriculture and industry by supplying plants needed to meet the growing demand. It has made significant contributions to the advancement of agricultural sciences recently, becoming an essential tool in modern agriculture. Biotechnology has been integrated into agricultural practices at an unprecedented rate. Tissue culture facilitates the production and propagation of genetically uniform, disease-free plantlets.

Scope and Employment Opportunities of Plant Tissue Culture Technician in India

More than 100 tissue culture companies are operating in India in private sectors. There are 11 biotech parks spread across the states which also run tissue culture laboratories. Besides, government research organizations, states and central universities also have tissue culture setup. The success of tissue culture lab depends on the tissue culture technician. The potential role of a technician in tissue culture lab is to:

- Initiate tissue culture from explants.
- Multiply already established culture.
- Prepare Culture medium.
- Washing culture vessels.
- Rooting the plantlets grown in tissue culture via *in vitro* or *ex vitro* technique.
- Hardening and acclimatization of rooted plantlets in green greenhouse.
- The technicians are expected to follow Standard Operating Procedures (SOPs)to keep exact, detailed records and collect data.
- They are also responsible for maintaining an inventory of chemicals, glassware and other supplies needed for a large-scale commercial laboratory

Employment Opportunity

The commercial plant tissue culture industry has grown significantly over last 30 years. The output has grown from 5 to 500 million plants a year.

The Career options in the field of Plant Tissue Culture can be:

- Agricultural technician
- Greenhouse or field technician
- Laboratory technician

- Plant tissue culture technician

In India, there are several employment opportunities for individuals with expertise in plant tissue culture. Plant tissue culture technicians can work in the Research and Development (R&D) laboratories focusing on developing new plant varieties, and improving crop yield through tissue culture techniques. Plant tissue culture technicians are needed for developing and maintaining cultures for various purposes.

Many research institutes research crop improvement, disease resistance, and other aspects of plant biology. Seed companies also work on this technique for mass production of disease-free and genetically uniform planting material. Pharmaceutical industries use plant tissue culture to produce bioactive compounds. Plant Tissue Culture Technicians are needed to manage and maintain tissue culture facilities and can serve in such organizations. Technicians can also work in quality control roles, ensuring the consistency and purity of tissue cultures produced, as well as adherence to regulatory standards.

Present scenario of Plant Tissue Culture Industry in India

The demand for superior quality disease-free clones of plants in various core sectors of agriculture like horticultural, ornamental plants, agro-forestry etc., always exists. Commercial tissue culture in India was first initiated way back in 1987 when a commercial unit for large-scale production of cardamom was established by NV. Thomas & Co. Ltd in Kerala. It is estimated by The Biotech Consortium (India) Limited that there are about 125 commercial plant tissue culture units with around 300 million plantlets produced every year from each unit. Most of these units are in Andhra Pradesh, Tamil Nadu, Maharashtra, Kerala and Karnataka. Major companies like Jain Irrigation Systems, Tata Tea, Hindustan Unilever, Nath Seeds, Unicorn Biotech, Cadilla etc. have been doing multi-million-dollar business for the past three decades in the field of plant tissue culture.

150+ projects related to plant tissue culture have been approved by The Ministry of Science and Technology in 80 different Universities and Research Institutes. Agriculture is one of the most profitable sectors due to the commercialization of PTC. The global market for PTC is projected to reach around 900 million USD by the year 2030.

Some of the plant species in which Commercial Micropropagation has successfully been employed are given below:

- **Fruits:** Bananas, Grapes, Strawberries, apples, citrus, pineapple, cashews, *sapota* and watermelon.
- **Cash Crop:** Sugarcane, potato and tapioca.

- **Spices:** Turmeric, ginger, vanilla, cardamom and clove.
- **Medicinal Plants:** Aloe*, Stevia, rosemary, tulsi, geranium, patchouli.*
- **Trees:** Teak, Populus, bamboo, pine, red sanders, eucalyptus.
- **Biofuel:** *Jatropa, Pangomia.*
- **Ornamentals:** *Saintpaulia, Gerbera, dracaena, carnation, roses, lily, cymbidium, cactus* etc.

Government Schemes and incentive in plant tissue culture industry are supported by many funding agencies like the Department of biotechnology (DBT), National Horticulture Board (NHB), Ministry of Agriculture and Farmers Welfare, and Ministry of AYUSH, etc.

A combined effort in terms of supportive policies from various departments of the Government of India has inspired entrepreneurs and technocrats to establish more commercial units of the plant tissue culture industry with immense commercial potential.

The major consumers of tissue culture-raised plantlets are the State Agriculture Department, Agri Export Zones (AEZs), State agencies such as Spice Board, the sugar industry and private farmers. The paper industry, medicinal plant industry and State Forest Departments are using tissue culture-raised plants at a limited scale. Also, several progressive farmers and nurseries in the states are the major consumers of tissue culture plantlets, particularly for flowers, bananas, sugarcane and medicinal plants.

Future Prospect of Plant Tissue Culture Industry

Biotechnology is progressively accepted as a strong and pervasive force that can mainly improve agricultural production and labour use. Plant Tissue Culture has become an integral part of plant breeding and is involved in the rapid mass multiplication of improved plants. Earlier, introducing new varieties to the market took several years, due to the slow multiplication rate *in vitro*. Micropropagation can speed up the process of bringing new varieties to the seed chain. In India itself, more than 100 plant species have been regenerated using plant tissue culture with the capacity of more than 350 million plants per annum. Plant tissue culture in future will remain a perpetual source for the production of industrially important bioactive compounds. Globally there is a shift towards plant-based products and drugs. However, the supply of plants is limited and the rare/threatened plants are even banned for commercial purposes. Thus, PTC will play a significant role in the future development of commercial endeavours.

Recombinant DNA technology also requires plant tissue protocols for its success. In the future, genome editing technology for the improvement of plants will be commonly used. Plant tissue culture will remain the core of all

genetic engineering research. Genetically Modified (GM) crops like Golden Rice, BT cotton, and *FlavrSavr* tomato require plant tissue culture protocol for the transfer of genes. With increasing population and erratic climate changes, tissue culture techniques have become more relevant**.**

CHECK YOUR PROGRESS

A. Fill in the blanks

1. The global market for plant tissue culture is valued to reach around _____ by 2030.
2. Plant tissue culture is a technique used to propagate plants and produce _____ plant materials.
3. _____ The estimated number of commercial PTC units in India is around _____ .
4. Fruit crops that can be propagated through PTC include banana, grapes, and _____ .
5. PTC can be used to cultivate plants for the_____ industry.

B. Multiple choice questions

1. Where are most commercial plant tissue culture units located in India?
   a) Gujarat and Rajasthan
   b) Punjab and Haryana
   c) Tamil Nadu and Kerala
   d) Uttar Pradesh and Bihar
2. Which government agency provides support to the Plant Tissue Culture industry in India?
   a) Ministry of Education
   b) Ministry of Defense
   c) Department of Biotechnology (DBT)
   d) Ministry of Transportation
3. Which sector has experienced significant growth due to the commercialization of plant tissue culture?
   a) Automotive
   b) Information Technology
   c) Agriculture
   d) Healthcare

4. What is the role of a Plant Tissue Culture technician?
   a) Cultivating fish
   b) Propagating plants in a laboratory
   c) Growing plant in field
   d) Designing buildings
5. Which of the following is NOT a crop commonly propagated through commercial micropropagation?
   a) Maize
   b) Banana
   c) Sugarcane
   d) Pineapple

C. Subjective questions

1. Explain the basic principles of plant tissue culture.
2. Explain different types of plant tissue culture techniques and their applications.

Module 2

Laboratory Setup and Equipment for Plant Tissue Culture

Module Overview

Tissue culture is the art and science of multiplying plantlets through any part of the plants *viz.*, shoot, root, leaf, anther, seed, ovules etc. Setting up a plant tissue culture laboratory requires careful planning and consideration of key aspects like space, equipment, materials, protocol etc. Tissue culture is a multi-step procedure in which tissues are grown on a specialized nutrient medium under *in vitro* conditions. It offers several advantages over the conventional system of propagation such as a high rate of multiplication, the low cost of production and independence from season. Tissue culture technology is being extensively used for large-scale production of elite planting material of desired characteristics. Many crops are being propagated commercially through tissue culture. Tissue culture technology becomes increasingly important for large-scale production of high-quality planting material with desired traits. Production of high-quality planting materials needs well-equipped laboratory setups. These setups must maintain strict aseptic conditions to prevent contamination and ensure the success of tissue culture procedures.

Learning Outcomes

After completing this module, you will be able to:

1. Understand the essential components required for setting up a plant tissue culture laboratory.
2. Identify important laboratory equipment and explain the sterilization process to maintain a contamination-free environment.

Module Structure

- Session 3: integrated disease management
- Session 4: weed management

Session 1: Laboratory Setup and its Component

Tissue culture laboratory setup is not only capital intensive but also requires an understanding of the subject. The first step of setting up of plant tissue culture lab is to select a suitable location. The lab should be spacious and well-ventilated, allowing free movement of equipment and personnel. It should also have adequate electrical power and water supply, as well as proper

temperature control. Additionally, the lab should have proper storage facilities for the planting materials used in tissue culture.

Setting-up a Tissue Culture Laboratory

Human Resources

Human Resources is one of the important parts of successful operation of a tissue culture facility. A number of managers and technicians will vary depending upon the size and production capacity of the tissue culture facility. Usually, there is one Manager or in charge of the lab who is responsible for the recruitment of human resources, purchase of equipment and glassware, development and implementation of the standard operating protocol in the lab, planning for production of tissue culture facility and implementation of quality system and procedures. Usually, there are four managerial positions in a standard tissue culture lab. The manager (Research & Development /Quality Management) is responsible for the development or standardization of tissue culture protocols, selection of mother plants, maintenance of records, virus indexing or genetic fidelity testing of plants and calibration of equipment. The production Manager looks after the planning and execution of the production of tissue culture plants and the overall hygiene of the tissue culture facility. Green House manager looks after the hardening facility which includes greenhouse and secondary hardening for data and quality management at the nursery stage. The marketing manager looks after the marketing/shipment of plants provides a package of practice to farmers, demonstrates tissue culture technology at farmers' field and takes feedback from customers. The hierarchy of manpower is depicted in Figure 2.1.

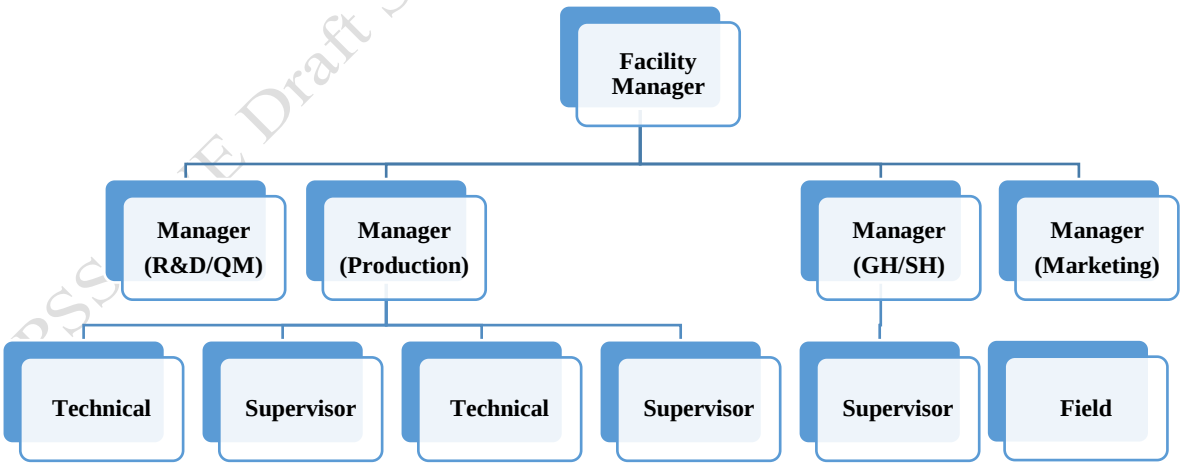

Fig: 2.1 Human resource structure in a tissue culture lab

Laboratory layout

Location

Tissue culture laboratories should be situated near the cities where water, electricity and human resources are available. Tissue culture laboratories should not be situated in residential areas as laboratories generate hazardous waste which needs to be disposed of safely. The size of the laboratory depends upon the production target. However, an average tissue culture laboratory usually spreads over 450 to 500 m^2 and can produce 05-10 lakh plants per annum. A laboratory situated in a milder climate is more suitable as it will reduce the burden of electricity charges. The site of the lab should be well-connected with sufficiently wide roads for tractor-trolley and trucks. Nearness to railway stations or airports is advantageous for the shipment of tissue-cultured plants.

Laboratory design

A standard tissue culture laboratory should be well-planned with proper drainage, safe electric wiring and a cooling system. The entrance of tissue culture labs should be regulated by a double door. The facility should be labeled as "**No Entry without Permission**". Tissue culture laboratory may be single- or multi-story depending on the space and requirement. The tissue culture labs should be fitted with solar panels to utilize natural energy resources to reduce production costs. Modern tissue culture laboratories are fitted with sensors (door sensors), data loggers, temperature sensors, fungal colony counters, ozone generators and robotized mopping and cleaning. The tissue culture facility should either be provided with a centrally air-conditioned plant providing ducted cool-air filtered through HEPA filters (0.22 µm) @ two air changes per minute or with individual air-handling units provided with High-Efficiency Particulate Air (HEPA) filters (0.22 µm) feeding specific areas of the facility. The laboratory should be equipped with a fire alarm and fire extinguisher. National Accreditation Board for Testing and Calibration Laboratories (NABL) accreditation of the lab is recommended.

The design of a tissue culture lab is critical to ensure that the environment is conducive to the growth and development of the plantlets, while also maintaining a strictly sterile environment and preventing contamination. The following points should be considered while constructing a lab:

- **Hygienic environment**: To reduce contamination of culture, the tissue culture lab should maintain a high degree of sterile airflow. The air conditioners should have a High-Efficiency Particulate Air (HEPA) filtration system to remove airborne particles to maintain low particle counts.

- **Illumination**: The lab should have specialized lighting to provide the necessary light spectrum for plant growth. LED lighting is commonly used to provide the required spectrum and efficient energy.
- **Environment control**: The lab should have temperature and humidity control systems to maintain optimal conditions for plant growth. The temperature and humidity levels should be monitored and adjusted as needed.
- **Workstations**: The lab should have a dedicated Laminar Air Flow Cabinet for different tissue culture stages. These workstations should have aseptic surfaces, laminar flow hoods, and other necessary equipment for tissue culture work.
- **Storage facilities**: The lab should have storage facilities for plant material, media, and other necessary supplements. These storage facilities should be designed to maintain a sterile environment to prevent contamination.
- **Waste management**: The lab should have a waste management system to dispose of contaminated materials for prevention of contamination.
- **Safetymeasures**: The lab should have Fire alarms and Fire extinguisher in each room to mitigate fire accident in the lab. The lab should have sufficient Personal Protective Equipment (PPE, gloves, shoe cover, headgear etc.), emergency response plans, and safety training for lab personnel.

Important Component of a Tissue Culture Lab

The tissue culture lab is a complex building which has several important structures. A tissue culture laboratory should be divided into two separate areas connected with an outer passage.

- Aseptic Area
- Non-aseptic area

Aseptic Area

The aseptic area of the lab is the main component where major tissue culture operations are conducted. This part should be free from any microbial presence. This is a highly restricted area where only an authorized person should enter. The entry of the aseptic area should have a double door, which is followed by a changing room and an ultraviolet room. The operators or technicians should enter through double door to change their day-to-day clothes and remove their phones, wallets and all jewellery. One should always wear laboratory gear then should proceed to the aseptic area. Before entering into the inoculation room, they should cross the fan. The aseptic area is divided into four parts:

1. Inoculation room
2. Growth room
3. Media preparation room
4. Media storage room

1. Inoculation room

The Inoculation room is the main component of a tissue culture lab, fitted with laminar air flow cabinets for culturing the plant tissues (**Figure 2.2**). The size of the Inoculation room depends upon the production target of the company. The floor and wall of the room should be tiled and adjacent to the culture area covered with a lane so that immediately after inoculation, cultures can be transferred to the growth room. The inoculation room should have two doors, one for entry (fitted with an air curtain) and one for transporting culture bottles to the growth room (air curtain). The temperature should be 25± 2°C. The inoculation room, media storage room and growth room will be maintained at a minimum sterile environment level of class 100,000 and provided with positive pressure. The inlets of receiving air should be located at the top side or shelf level and the outlets of exhausting air should be located close to floor level on opposite side to facilitate uniform air circulation and minimize contamination. The inoculation room should be cleaned with a vacuum cleaner and the floor area should be daily mopped with disinfectant.

Fig: 2.2: Inoculation room

2. Growth room

The growth room or growth chamber is the second most sensitive area where atmost hygiene is maintained. The environmental conditions like temperature, humidity, intensity and duration of light are regulated as per the requirement of the plant species. The main door of the lab should be connected through dust-proof air curtain. The wall of this room should be thick or insulated. The roof should be covered with a false ceiling. The floor and walls of this room should be furnished with tiles. The growth chambers house several culture racks therefore, the height of the roof should be such that culture racks can move freely. The temperature of the room should be maintained at 25± 2 °C through heap filter-mediated cool air. The growth room door should be big enough to facilitate the movement of the trolley. The data loggers must be fitted in the room along with the colony counter to maximize precautions when microbial continuation is at its peak. There can be several growth rooms (**Figure 2.3**) in a lab depending upon the production targets.

Figure 2.3: Growth room

3. Media preparation room

The media preparation room is situated in the central portion of the laboratory. The size of this room is slightly bigger where equipment can be installed and technicians can work with ease. A long laboratory granite table top along with drawers should installed in the middle of the media preparation room. A sufficient storage facility should be available for keeping reagents, chemicals etc. This room should have the following equipment:

- **pH Meter**: To measure acidity or alkalinity of media so that pH can be adjusted.

- **Refrigerator**: To store growth regulators and temperature-sensitive chemicals.
- **Electronic balance:** To weigh chemicals in minute quantity.
- **Pan balance:** Used for weighing chemicals and gelling agents required in large quantities.
- **BOD incubator**: To maintain plant tissues at specific temperatures and light.
- **Distillation unit**: For the production of tissue culture grade water free from microbes and metals.
- **Hot plate magnetic stirrer:** Nutrient media is stirred using this equipment for homogenization.
- **Microwave oven:** Used to dissolve chemicals under high heat.
- **Incubator shaker:** It is used to keep cultures under specific agitation in liquid solutions.

Media boiling unit: Nutrient media should be boiled after adding all the ingredients in the media boiling unit so that the gelling agent is mixed properly.

4. Media storage room

The media storage room is an important part of the tissue culture laboratory. It should be situated close to the Inoculation room so that media can be transported from the Media Storage room to the Inoculation room in a short period in a trolley. The temperature in this room should be moderate. The room should be fitted with GI racks for storage of medium. There should be provision of UV radiation inside the room to make it aseptic. The room should be fumigated regularly to avoid any microbial contamination.

Non-aseptic area

This is the second part of the tissue culture laboratory which should be placed 10-15 feet away from the aseptic area. This part is situated on the back side of the main laboratory. This part should have proper drainage, electrical connection and fire safety equipment. People working in this area should not enter into an aseptic area. The entrance for this area should be separate. This part of the laboratory should have the following rooms:

1. Autoclaving room
2. Store room
3. Washing room
4. Office room

1. Autoclaving room

The autoclaving room is an important part of the lab where nutrient media is being sterilized using an autoclave. This should be big with proper ventilation and a separate electricity connection. Double-door autoclaves are installed on one side (Loading Side) remain in the autoclave room and the delivery door is opened in the media storage room. The wall connecting both rooms should remain sealed. Small and medium-sized autoclaves can be installed in the autoclave room. Autoclaves are also used to sterilize contaminated cultures. The autoclave room should be connected to the washing room where contaminated/old culture bottles are washed. The standard operating protocol should be pasted on the wall of this room. A three-phase separate electricity connection should be laid with proper provision of earthing.

2. Washing room

This room should be provisioned with proper water supply and proper drainage. The wall and floor of this room should be furnished with tiles. An automatic washing machine with a dryer is used to wash culture vessels whereas, hand washing is practised in small labs. Standard Operating Protocol should be pasted on the wall of this room.

3. Office room

The office room keeps all records of tissue culture input, manpower engagements, demand of planting material and sales. The office room should be fitted with CCTV to keep watch on the inoculation room, autoclave room, media preparation room, washing room and growth room for day-to-day monitoring. Qualified Managers trained in tissue culture operations and sales should usually operate from here.

4. Storage room

This room is used to store consumables required for the tissue culture lab throughout the year. This room should have an iron almirah to store spirit, soaps, aprons, towels, wash brushes, fungicides, potting mixtures, poly bags etc.

Acclimatization

Tissue culture plants are produced in controlled environmental conditions using an artificial culture medium. When plantlets are removed from a controlled environment, they require to adjust to the open environmental conditions under various level of light intensity, temperature and humidity. Low nutrient availability and pressure of biotic threats or secondary things inhibited the growth and development of plantlets. Therefore, acclimatization is achieved in the protected structures designed for this purpose.

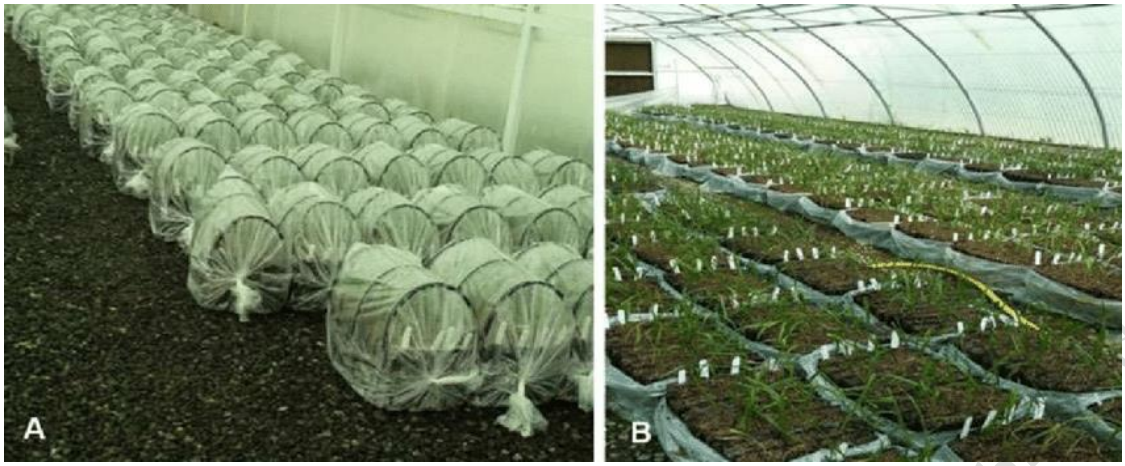

Fig: 2.4: Acclimatization of plantlets: Transition from lab to natural conditions

Primary hardening facility

A primary hardening polyhouse is a type of structure for the acclimatization of tissue culture plants to prepare them for the outdoor environment before they are transplanted into the field. Since tissue-cultured plants are supported by carbon and other major micro-nutrients during their growth phase in the laboratory, therefore, their photosynthesis apparatus is not fully functional. The acclimatization process helps the plants become stronger and more resilient to withstand the natural conditions. A primary hardening house is typically a semi-enclosed structure made of polyethylene or other similar materials that protect from harsh weather elements such as strong winds, excessive sunlight, or heavy rainfall. The polyhouse allows for natural light to penetrate while also maintaining a controlled environment for the plants as they transit from the controlled conditions of the tissue culture laboratory to the external environment. The structure should have adequate ventilation which is essential to prevent the buildup of excess heat and humidity inside the polyhouse, which can be detrimental to the plants. Ventilation openings or adjustable vents help regulate air circulation and maintain optimal growing conditions. Providing shade in the polyhouse helps protect the young tissue culture plants from direct sunlight, which can cause sunburn and stress. The water misting system within the polyhouse ensures that the tissue culture plants receive sufficient water to support their growth and development during the acclimatization process. The structure is fitted with a cooling pad, fans, heaters, or cooling systems to maintain an optimal temperature range for the plants. Maintaining suitable temperature levels is essential for the successful acclimatization of tissue culture plants.

Secondary Hardening Facility

A secondary hardening shade net house is a structure used to further acclimatize plants that have already undergone primary hardening in a

polyhouse. This phase of hardening helps plants adapt to outdoor conditions before they are transplanted into the field, ensuring their successful growth and development in the natural environment. The secondary hardening shade net house is typically constructed using a framework of poles or metal structures covered with shade netting material. The shade netting protects from direct sunlight, excessive heat, and other environmental factors while allowing air circulation and light diffusion to promote healthy plant growth. Different shading percentages (50%,75% or 80%) can be selected based on the specific light requirements of the plants. As discussed above, ventilation is essential to prevent the buildup of heat and humidity inside the shade net house, which can negatively impact plant growth. Ventilation openings, adjustable vents, or fans may be installed to regulate airflow and maintain optimal growing conditions for the plants. An irrigation system within the shade net house ensures that plants receive sufficient water to support their growth during the hardening-off process. Drip irrigation and overhead sprinklers may be used to provide consistent moisture to the plants. Temperature control mechanisms such as fans, evaporative cooling systems, or shade cloth can help regulate temperatures inside the shade net house and create a suitable environment for plant growth. Maintaining optimal temperature levels is crucial for the successful hardening of plants.

Preparation of Common Reagents Used in Plant Tissue Culture

Plant tissue culture involves the growth and maintenance of plant cells, tissues, and organs in an artificial environment. To carry out a successful tissue culture, several reagents are required. These reagents are instrumental in ensuring that the plant cells can grow and divide in a manner that closely mimics their natural environment. One of the most important reagents used in plant tissue culture is *Agar*. *Agar* is an ideal solidifying agent in the media. It is chemically inert and dissolves easily in warm water. Approximately, 7-8g *agar* is used in one-liter media. It solidifies upon cooling of media. The *Murashige* and *Skoog* (MS) medium contains inorganic salts and Fe-EDTA, providing essential minerals for plant growth, while vitamins support metabolic activities crucial for development. The composition of *Murashige* and *Skoog* Medium is as follows:

Table 1: Preparation of MS-Medium

Stock AMSMajor Salts	
Chemical	**Weight (g/l)**
KNO_3	19.0
$NH4NO_3$	16.5
$MgSO_4$ $7H_2O$	3.7
$CaCl_2$ $2H_2O$	4.4
KH_2 PO_4	1.7
Stock B-MS Minor Salts	
H_3BO_4	0.62
$MnSO_4$ $4H_2O$	0.22
Na_2MO_4 $2H_2O$	0.0025
$ZnSO_4$ $7H_2O$	0.86
$CuSO_4$ $5H_2O$	0.00025
$CoCl_2$ $6H_2O$	0.00025
Stock C- Fe-EDTA	
Na_2 EDTA	0.373
$FeSO_4$ $7H_2O$	0.278
Stock D. Vitamins	
Nicotinic Acid	0.005
Thiamine HCl	0.005
Pyridoxine HCl	0.001
Myo -inositol	0.1

ACTIVITIES

Activity- 1

Visit a nearby plant tissue culture lab and observe the arrangement of chemicals and equipment .

Material Required: Pen, pencil, notebook, etc.

Procedure

1. Visit the nearby Plant Tissue Culture Lab.
2. Observe and note down the following:

   a) Enlist the chemicals used in the plant tissue culture laboratory.

b) Arrangement of chemicals as per utility
c) See the various compartments of the tissue culture lab like the incubation chamber, growth room, media preparation and storage place.
d) Discuss and note down the safety measures.
e) Any other observations.

If you have any queries, please discuss them with the lab manager.

CHECK YOUR PROGRESS

A. Fill in the blanks

1. Aseptic area of a lab is free from_____.
2. A _____ is used for the acclimatization of tissue culture plants to prepare them for the outdoor environment before they are transplanted into the field.
3. Environmental conditions such as temperature, humidity, and light are maintained in _____ for optimal plant growth.
4. _____ is the main component of the tissue culture lab which is fitted with Laminar Air flow cabinets for culturing the plant tissue.
5. Nutrient media for plant growth is prepared in the_____ room.

B. Multiple choice questions

1. Temperature of growth room should be:
   a) 28±2°C
   b) 25±2°C
   c) 32±2°C
   d) 35±2°C
2. Entry of aseptic area should have:
   a) Double door
   b) Single door
   c) Triple door
   d) No door
3. Evaporative cooling is used in:
   a) Media room
   b) Autoclaving room
   c) Washing room
   d) Secondary hardening facility

4. Which of the following is NOT a component of the aseptic area?
   a) Growth room
   b) Media preparation room
   c) Washing room
   d) Inoculation room
5. Tissue culture lab should be situated near:
   a) Village
   b) Forest area
   c) City
   d) Mountains
6. The primary function of agar in plant tissue culture media is:
   e) Providing nutrients
   f) Regulating pH
   g) Solidifying the medium
   h) Controlling temperature

C. Subjective questions

a) Describe the aseptic area of the tissue culture lab.
b) Describe the human resource structure in a tissue culture lab.
c) Describe stock solutions of MS medium.

Session 2: Laboratory Equipment and Sterilization Process

Tissue culture operations are carried out by specialized equipment such as an autoclave, laminar air flow cabinet, pH meter, magnetic stirrer hot plate, culture racks, BOD incubators, Incubator shakers, electronic balance etc. It is important to know how equipment works and what precautions need to be taken to operate different equipment. Maintaining the sterility of the lab is essential for successful tissue culture. Some of the common techniques which are employed in tissue culture labs are steam sterilization (Autoclave), chemical sterilization (Potassium dichromate, formaldehyde), boiling and cold filtration. Explant cleaning is also a very important aspect which requires chemicals like mercuric chloride, ethanol, hydrogen peroxide and sodium hypochlorite. The type and concentration of this disinfectant depend upon the type of explants used.

Equipment Used in Tissue Culture Laboratory

1. Autoclave An autoclave is a device used to sterilize nutrient media, glassware, equipment, and other materials by subjecting them to high-pressure steam at temperatures typically exceeding 121°C (250°F) for a specific amount of time. The high pressure and temperature inside the autoclave ensure that all microorganisms, including spores, are destroyed, making the items safe for use in tissue culture applications. Vertical and horizontal autoclaves are used in tissue culture facilities. They can be powered by electricity or gas. (Figure 2.5).

Fig: 2.5: An autoclave used to sterilize nutrient media, glassware, equipment, and other materials

2. pH Meter

A pH meter is an instrument used to measure the acidity or basicity of a solution. The pH scale ranges from 0 (most acidic) to 14 (most basic), with 7 being neutral. A pH meter consists of an electrode (usually glass or plastic) that is inserted into the solution being tested and an electronic display that shows the pH value. The electrode detects hydrogen ions (H+) in solution which are responsible for acidity or basicity. The pH of the standard tissue culture medium is kept at 5.8 (Figure 2.6).

Fig: 2.6: 9A Ph Meter used to measure the acidity or basicity of a solution

3. Laminar Air Flow Cabinet

To create a covered, contamination-free platform for the inoculation of explants, laminar air flow cabinets are used which provide sterile air through HEPA filters. All the particles and microbes are captured in the filter. The hood is connected through a 0.25-0.5 HP motor which sucks air from the environment via the primary filter and passes it through a heap filter (0.2 micron). LED lights and UV lights are fitted inside the chambers. The manometer is also fixed to see the pressure of the chamber. A Laminar air flow cabinet is one of the most important instruments for sub-culturing and inoculation of explants. Usually, a glass bead sterilizer is kept inside the laminar air flow for sterilization of forceps, spatula and dissecting razors. Sometimes spirit lamps are used for sterilization of forceps etc. inside laminar

air flow (figure 2.7). Two types of laminar airflow viz., vertical and horizontal are available in the market.

Fig: 1 Laminar Airflow

4. Electronic Balance

An electronic balance is a precise weighing instrument used to measure the mass of an object with high accuracy. It consists of a platform or pan where the object is placed, electronic sensors to detect the weight of the object, and a digital display to show the mass in units such as grams, or milligrams. Electronic balances play an important role in weighing chemicals particularly plant growth regulators in small amounts (Figure 2.8).

Fig: 2.8: An electronic balance used to measure the mass of an object with high accuracy

5. BOD Incubators

Biological Oxygen Demand (BOD) incubators provide a controlled environment to maintain a consistent temperature, pH, and dissolved oxygen level to ensure accurate test results. They are essential tools for tissue culture facilities specially if it is dealing with microbes. BOD incubators (Figure 2.9) are also utilized for the growth of certain plants at specific temperatures.

Fig: 2 A BOD Incubator

6. Hot Air Oven

A hot air oven (Figure 2.10) is a laboratory instrument used to dry, cure, or sterilize various materials at a controlled temperature. It consists of a chamber with heating elements, a fan to circulate the air, and a thermostat to maintain a constant temperature. The materials to be treated are placed on racks or shelves inside the oven, and the door is sealed to prevent air from entering. The oven's temperature range can vary depending on the application, up to 300°C. Usually, culture bottles, pipettes, and glassware are sterilized in a hot air oven.

Fig: 2.10: 3 A Hot Air Oven

7. Magnetic Stirrer Plate

A magnetic stirrer plate is a tissue culture laboratory equipment designed to provide continuous stirring of liquids in test tubes, beakers, or flasks. It consists of a flat plate with multiple magnetic stirring bars embedded into it, which are activated by an external magnetic stirrer motor that rotates them at high speeds. The magnetic stirrer plate is commonly used in chemical reactions, mixing of solutions, and dissolution of solids in liquids. It ensures that the reactants are evenly distributed, preventing the formation of hotspots or sedimentation, which can lead to inaccurate results. The magnetic stirrer plate is available in various sizes and configurations, depending on the specific application requirements such as volume capacity, stirring speed range, compatibility with different types of containers, etc. Please mention the range of speed of the stirrer (Figure 2.11)

Fig: 2.11: 4 Magnetic Stirrer Plate

8. Microwave oven

A microwave oven (Figure 2.12) is an appliance that uses microwave radiation to heat solutions quickly and evenly. It consists of a metal enclosure with a turntable inside, which rotates the food as it is being heated. The microwave oven generates high-frequency electromagnetic waves that penetrate the food molecules causing them to vibrate rapidly, producing heat without directly touching the solution.

Fig: 2.12: Microwave oven

9. Tissue Culture-grade Water Distillation Unit

A tissue culture-grade water distillation unit is a specialized laboratory equipment used to produce ultra-pure water for tissue culture and other sensitive applications. This type of water purification system removes impurities and contaminants from water, ensuring that it meets the stringent quality standards required for molecular biology experiments. For lab experiments, the water distillation unit typically consists of a distillation chamber where water is heated to generate steam, which is then condensed back into liquid form, leaving behind impurities and contaminants. The distillation process effectively removes organic and inorganic compounds, ions, bacteria, and other impurities, resulting in high-purity water suitable for tissue culture applications. Tissue culture-grade water distillation units are designed to produce water with extremely low levels of trace contaminants. These units often incorporate additional purification technologies such as carbon filtration, reverse osmosis, or UV sterilization to further enhance the water quality (Figure 2.13).

Fig: 5 Tissue Culture- grade Water Distillation Unit

10. Incubator Shaker

An incubator shaker is a laboratory instrument that combines the functions of an incubator and a shaker in a single device. It is commonly used in microbiology, biochemistry, and tissue culture research to cultivate and maintain bacterial, fungal, or plant cell cultures under controlled temperature, humidity, and agitation conditions. The incubator shaker consists of a temperature-controlled chamber that can maintain a specific temperature range, typically between 2-45°C, to support the growth of microorganisms or cells. The chamber is equipped with a shaking mechanism that provides gentle agitation to the culture medium, preventing sedimentation of cells and ensuring uniform nutrient distribution. The incubator shaker can be programmed to operate in different modes, such as continuous shaking, intermittent shaking, or no shaking, depending on the specific application requirements. It can also be equipped with various accessories, such as gas inlets, pH probes, and oxygen sensors, to monitor and control the culture environment. The incubator shaker is particularly useful for large-scale fermentation and cell culture applications, where the combination of temperature, humidity, and agitation control is critical for optimal cell growth and product yield. It allows for efficient and consistent culture production, reducing the risk of contamination and improving reproducibility of results, making it a valuable tool for researchers in various fields of life sciences and biotechnology research (Figure 2.14).

Fig: 2.14: Incubator Shaker

11. Refrigerator

A refrigerator is a laboratory equipment designed to maintain a consistent temperature below room temperature, typically between 0°C and 8°C (32°F to 46°F), to preserve plant growth regulators, nutrient stock solutions, vitamins etc. In a laboratory setting refrigerators are used to store biological samples ensuring their stability, potency and safety for future use (Figure 2.15).

Fig: 6 Refrigerator

12. Tissue Culture Rack

A tissue culture rack is a specialized piece of laboratory equipment used for the storage and incubation of tissue culture plates or flasks in a cell culture laboratory. These racks are designed to provide a convenient and organized way to store multiple culture vessels while optimizing space and facilitating easy access to individual cultures. Tissue culture racks typically consist of a metal frame with several shelves or tiers that can hold tissue culture plates, flasks, or other vessels used for growing cells in a sterile environment. The shelves are usually adjustable or removable to accommodate different sizes of culture vessels and allow for customization based on the specific needs of the user. The racks are often designed with features that promote proper air circulation around the culture vessels, which is important for maintaining optimal conditions for cell growth and preventing contamination. Some racks may also include built-in temperature control options, such as heating or cooling elements, to provide a stable and controlled environment for the cultures (Figure 2.16).

Fig: 2.16: Tissue Culture Rack

Importance of sterilization of Lab and Lab Equipment

Sterilization is the process of killing all microorganisms (bacterial, viral, and fungal) with the use of either physical or chemical agents. Sterilization in the tissue culture laboratory is essential for culture media, glassware and sometimes equipment.

Sterilization Process

Maintaining the sterility of the tissue culture lab is essential for successful cell culture. Autoclaving is a commonly used sterilization method for equipment and materials. It involves using pressurized steam at high temperatures to kill microorganisms. Some equipment and materials can be sterilized using certain chemicals, such as potassium dichromate or formaldehyde. Filtration techniques, such as membrane filters or sterile filters, can be employed to remove microorganisms from liquids or gases.

Cleaning and Washing of Explants

The explants should be collected either in the morning or evening. Care should be taken to excise healthy and actively growing tissue from the plant. The explants immediately after excision should be kept in cold water fortified with 1 drop of detergent. Thereafter explants should be brought back to the laboratory and should be prewashed under running tap water for 30 minutes. Then explants should be kept in a washing solution containing fungicide, antibiotic and detergent for one hour under agitated conditions (Shaker). After that, the explants are washed with water to remove the chemicals.

The washed explants are brought back under laminar airflow and disinfected using sodium hypochlorite, hydrogen peroxide or mercuric chloride depending upon the explants. Sometimes the explants are dipped in ethanol for disinfection. The process of cleaning and washing explants is outlined in the flowchart below:

Disinfection of Glassware

Setting up a tissue culture lab requires proper disinfection of lab glassware to ensure a clean and sterile environment. Disinfection is crucial for maintaining cell cultures and ensuring their viability. To disinfect lab glassware, several methods can be used.

- **Autoclaving:** Autoclaving is a commonly used method for sterilizing laboratory glassware. It involves exposing the glassware to high-pressure steam for a specified duration to kill microorganisms. Autoclaves are readily available in most laboratories and are commonly used to disinfect various equipment, including glassware.
- **Chemical Disinfectants:** Chemical disinfectants can also be used to disinfect lab glassware. These disinfectants work by destroying the

microbial cell wall, preventing the microorganisms from replicating (Potassium dichromate). Chemical disinfectants are commonly used for items that cannot withstand high temperatures, such as plastic containers or glass pipettes.

- **Dry Heat Sterilization**: Dry heat sterilization is another method for sterilizing lab glassware. It involves exposing the glassware to high 160-170 °C for 1 hour, killing microorganisms. This method is suitable for glassware that cannot be autoclaved or is not heat-resistant.
- **Sterilization Pouches:** Sterilization pouches are a convenient option for disinfecting lab glassware. These pouches contain a sporicidal agent that kills microorganisms. The glassware is placed into the pouch, which is then sealed and autoclaved or irradiated.
- **Boiling Water:** Boiling water is a simple method for disinfecting lab glassware. It involves immersing the glassware in hot water for a specified duration, which helps kill microorganisms. This method is especially suitable for glassware that cannot be autoclaved or disinfected using chemical disinfectants.
- **Cleaning and Drying:** The final step in the disinfection process is cleaning and drying the glassware. This helps remove any remaining debris or contaminants that may interfere with the sterilization process. Washed culture bottles /glassware can be dried in hot air oven at 60 °C temperature.

Precautions to be taken for Equipment

Equipment should have a logbook where the user must enter the time of usage and working condition at the end of operation**.** The SOP for equipment should be pasted nearby so that it operates in proper condition**.** Certain equipment such as pH meters should be calibrated from time to time. Costly equipment such as incubator shakers, water purification systems, autoclaves and laminar air flow cabinets should have an Annual Maintenance Contract (AMC) so that their efficiency is maintained.

Disposal of Tissue Culture Waste

In the process of tissue culture, waste management is a critical aspect that often unnoticed. Understanding the types of tissue culture waste and appropriate disposal methods is crucial for maintaining both environmental stewardship and laboratory safety.

Type of Tissue Culture Waste

- **Liquid Waste:** This includes culture medium, washing solutions, and reagents used in tissue culture experiments.

- **Solid Waste:** This includes disposable items such as culture dishes, pipette tips, and gloves.

Waste Management

The tissue culture lab produces both solid and liquid waste. Proper disposal of lab waste is mandatory. It is important to label all the liquid waste with origin. The liquid waste should be stored in the container and should be disposed of using incinerators, chemical treatment or through authorized waste management companies. Solid waste should be collected in appropriate containers or waste bags. Solid waste can be stored in designated containers or bins until it is ready for disposal. Solid waste should be disposed of in a designated waste disposal area. This may include regular waste collection, recycling, or incineration.

Safety Precautions

When handling tissue culture waste, the following safety precautions should be followed:

- Wear appropriate Personal Protective Equipment (PPE), such as gloves, lab coats, and safety glasses.
- Follow proper hygiene practices, such as hand washing after handling waste.
- Avoid direct contact with waste whenever possible.
- Ensure that waste containers are properly labelled and sealed to prevent contamination.
- Follow established protocols and guidelines provided by the laboratory.

ACTIVITIES

Activity- 1

Visit nearby plant tissue culture lab and enlist common equipment used.

Material Required: Pen, pencil, notebook

Procedure

1. Visit a nearby tissue culture lab and note down the following:
   - Name of equipment and its use
   - Name of glassware and its use
2. Demonstrate Waste management in a lab.

If you have any queries, discuss them with the lab manager.

CHECK YOUR PROGRESS

A. Fill in the blanks

1. _____ is used to sterilize nutrient media.

2. The temperature of steam inside the autoclave is _____.
3. pH meter is used to measure_____ of a solution.
4. The pore size of HEPA filter is _____ micron.
5. Membrane filters are employed to remove_____ from liquids or gases.

B. Multiple choice questions

1. Which equipment is used for the inoculation of explants/tissue?
   a) Autoclave
   b) BOD Incubator
   c) Incubator Shaker
   d) Laminar Air Flow Cabinet
2. Continuous stirring of liquid can be achieved by:
   a) Laminar air flow cabinet
   b) Refrigerator
   c) Magnetic stirrer plate
   d) Hot air oven
3. Incinerators are used for:
   a) Media preparation
   b) pH measurement
   c) Production of distilled water
   d) Waste management
4. Mercuric chloride is used for
   a) Increasing the pH of a solution
   b) Gelling of media
   c) Disposal of waste
   d) Disinfect of explants
5. Which of the following is the primary purpose of BOD incubators?
   a) To regulate humidity levels
   b) To maintain temperature, pH, and dissolved oxygen level
   c) To promote bacterial growth
   d) To sterilize samples

C. Subjective questions

a) Describe the method of waste management in the tissue culture lab

Module 3

Preparation and Storage of Culture Media for Plant Tissue Culture

Module Overview

The growth, development, and morphogenesis of an explant in culture are highly influenced by its genetic makeup, surrounding environment, and the composition of the culture medium. The selection of suitable nutrient media is crucial for successful organogenesis. The development of a basic culture medium is determined by the nutritional needs of plants in soil, as well as nutrient solutions used for plant culture. Culture media play a key role in the *in vitro* growth and morphogenesis of plant tissues. The success of plant tissue culture depends on selecting a nutrient medium with the right concentrations of macro and micronutrients necessary for the growth and maturation of the plant source or tissue. Since the nutrient requirements of plant tissues vary, there is no single medium suitable for all plant tissue growth. Therefore, finding the appropriate medium for a particular plant often involves a trial-and-error approach.

Learning Outcomes

After completing this module, you will be able to:

1. Identify different types of tissue culture nutrient media and their specific applications.
2. Understand the general methodology for preparing culture media in a laboratory setting.
3. Learn the process of preparing buffers and solutions essential for plant tissue culture.

Module Structure

Session 1: Types of Tissue Culture Nutrient Medium

Session 2: General Methodology of Media Preparation

Session 3: Preparation of Buffers and Solutions

Session 1: Types of Tissue Culture Nutrient Medium

Media constituents

Culture media used for *in vitro* cultivation of plant cells consists of a complex mixture of inorganic nutrients (macronutrient and micronutrients), organic nutrients (vitamins and amino acids), Plant Growth Regulators (PGRs), solidifying agents and antibiotics.

1- Inorganic Nutrients

1.1- Macronutrients

These are the inorganic salts required in adequate quantity. Micronutrient includes nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulphur (S) for plant cell or tissue growth. These are incorporated into the medium in compound form as given in Table 3.1.

1.2- Micronutrients

Micronutrients are the second most nutrient source required in small quantities. They are crucial for the preparation of culture medium for growth and development under *in vitro* conditions. These are Boron (BO_3^{3-}), Manganese (Mn_2^+), iron (Fe_2^+), Zinc (Zn_2^+), Copper (Cu_2^+), Molybdenum (MoO_4), Cobalt (Co_2^+) and Iodine (I^-). To increase iron availability across a wide range of pH, chelated forms (Fe-EDTA) or equimolar quantities of chelating agents (e.g. Na_2EDTA) are added with the iron salt ($FeSO_4.7H_2O$). The micronutrients are part of the enzyme systems in plants. They play important roles in photosynthesis, nitrogen fixation and protein synthesis.

2. Organic Nutrients

1.1- Vitamins and Amino Acids

Vitamins are essential for proper plant growth and development of plant tissues. They are catalytic and used as a coenzyme to accelerate a variety of metabolic processes. The vitamins used generally from the B group, including thiamine (vitamin B_1), nicotinic acid (vitamin B_3, also known as niacin) and pyridoxine (vitamin B_6). Thiamine, nicotinic acid, and pyridoxine are supplied in hydrochloride (HCl) form. *Myo-inositol*/meso-inositol is incorporated in a medium in a higher amount (100 mg L^{-1}). Of these, thiamine and *Myo-inositol* are considered essential ingredients in tissue culture media.

In amino acid, Glycine is commonly used in plant tissue culture media. Other amino acids, such as L-glutamine, asparagine, serine, and proline are also sometimes added to the medium. These act as the organic source of reduced nitrogen.

3. Plant Growth Regulators (PGRs)

The incorporation of Plant Growth Regulators (PGRs) into a basal nutrient medium is essential to trigger various types of growth and differentiation responses. External supplementation of plant growth regulators is generally required to elicit desired responses. The type and concentration of PGRs used in a medium depends on the types of plant tissues and plant species. The stage of culture like axillary bud proliferation, callus induction, shoot differentiation/multiplication, somatic embryogenesis, rooting, etc. may also

require various concentration levels of the PGRs. The PGR concentrations are commonly expressed as mg L^{-1} which represents the actual amount of the chemical employed during media preparation.

3.1 *Auxin*

Auxins play a crucial role in plant development, including stem/internode elongation, apical dominance, tropism, abscission, and rooting. These are employed in tissue cultures to promote cell division, cell differentiation, organogenic and embryogenic differentiation. *Auxin* promotes root initiation at lower concentrations while causing callusing at higher concentrations. The commonly used auxins in plant tissue culture are: 2,4-dicholorophenoxyacetic acid (2,4-D), α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), and indole-3-butyric acid (IBA). In addition, naphthoxyacetic acid (NOA), 2,4,5-tricholorophenoxyacetic acid (2,4,5-T), Dicamba (DCA), and Picloram (PIC) have been also used.

Inodel-3 Acetic Acid is light light-sensitive and readily degradable type of auxin while the other auxins are synthetic and chemically stable. IAA, NAA and IBA are generally used auxins in shoot multiplication and rooting culture. The combination of IBA, NAA, and IAA with Cytokinin promotes shoot proliferation. For the promotion of somatic embryos in dicots, 2, 4-D is generally used while, monocots need DCA, and PIC.

Note: The auxins, NAA and 2,4-D are stable and can be stored at 4°C for a few months and longer durations, these can be stored at -20°C. IAA should be prepared afresh each time during media preparation; however, it can be stored in an amber bottle at 4°C for up to a week.

3.2 Cytokinins

Cytokinins are naturally synthesised by plants as N6-substituted adenine. They are important constituents of nucleosides and nucleotides. In tissue culture, it is essential for cell division, inducing differentiation of adventitious shoots from callus/organs, multiplication of shoots by releasing the axillary buds from apical dominance, and retard root formation. The commonly used *cytokinins* in tissue culture are 6-benzylaminopurine (BAP), 6-furfurylaminopurine (Kinetin), 2-isopentenyl-adenine (2iP) and 6-(4-hydroxy-3-methyl but-2-enyl amino)-purine (Zeatin).

3.3 Gibberellins

There are more than 20 types of gibberellins have been identified and GA_3 is the most preferred in plant tissue culture. *Gibberellins* are used to stimulate shoot/internode elongation, attain normal plant development from *in vitro* adventive embryos, influence seed development and overcome dormancy. GA_3 is easily soluble in cold water. It is heat sensitive (90% activity lost after

autoclaving), therefore filter-sterilized and incorporated into an autoclaved medium after cooling.

3.4 Abscisic acid and Ethylene

Abscisic acid (ABA), a natural growth inhibitor, regulate somatic embryo development and maturation. In tissue culture, it improves somatic embryo formation by increasing desiccation tolerance and reducing precocious germination.

Ethylene (C_2H_4) is a unique gaseous hormone produced by ageing and stressed tissues. When exposed to heat, oxidation, sunshine, or ionising radiation, the medium's organic constituents produce ethylene. Generally, it inhibits differentiation and growth, however, it may enhance somatic embryogenesis. Ethylene synthesis can be promoted by 1-aminocyclopropane-1-carboxylic acid (ACC) or 2- chloroethylphosphonic acid (ethephon).

Although PGRs are the most expensive media constituents, they contribute a little towards its cost as these are required in very small quantities.

4. Solidifying Agents

Solidifying Agents provide a platform in plant tissue culture. They are gelling agents like *agar*, agarose, gellan gum, *isabgol*, or calcium alginate used to provide a platform for regeneration. A gelling agent should be inert, autoclavable, and liquid when hot, allowing for easy dispensing in culture vessels. The medium's semi-solid structure allows explants to be placed on its surface while maintaining aerated conditions.

- ***Agar*:** Derived from the genera *Gracilaria*, *Gelidium* and *Eucheuma* (red algae), *agar* is the most widely used gelling agent in tissue culture. *Agar* is a high molecular weight polysaccharide composed of galactose molecules that bind with water. The gel's firmness varies based on the agar concentration and the medium pH. It solidifies at 45°C and is often added to the medium at 0.8-1% (*w/v*). Agarose, consisting of β-D (1-3) and 3,6-anhydro-α-L(1-4) galactopyranose molecules, is obtained by purifying agar. Thus, it is costlier than agar and used in the range of 0.4–1.0% (*w/v*).
- **Gellan gum:** Derived from the bacteria *Pseudomonas elodea*, gellan gum is a linear polysaccharide and is available in the market with various brand names (e.g., Phytagel, Gelrite). It is a popular alternative to agar since it can be dissolved in a cold solution. It should be added to medium while continuous stirring to avoid clumping. It is preferred over *agar* owing to its low cost per litre of medium (0.1–0.2 % only) as well as for many other advantages.

- **Liquid Medium:** Liquid culture has several advantages over semi-solid medium, including faster growth rates, rapid nutrient uptake, and dilution of secreted growth inhibitors (phenolics) to minimise their negative effects on *in vitro* growth. The cost of a tissue culture protocol can be reduced by using a liquid culture system. For supporting the inoculum in liquid culture, a filter paper bridge (raft) can be employed while keeping the cultures on a gyratory/rotatory shaker for proper aeration.

5. Antibiotics

Antibiotics are substances produced by some microorganisms. They can suppress the growth of other microorganisms and eventually destroy them. It suppresses bacterial infections, mould and yeast infections in cell cultures, and eliminates agrobacterium species after the transformation of plant tissue.

6. pH of medium

The optimal pH range for most tissue cultures is in the range of 5.0 to 6.0. The pH generally falls by 0.3 to 0.5 units after autoclaving. Before sterilization, pH can be adjusted to the required optimal level while preparing the medium. Generally, a pH above 6.0 gives the medium a hard appearance, while a pH below 5.0 does not allow gelling of the medium. At pH higher than 7.0 and lower than 4.5, the plant cells stop growing in cultures. The composition of culture media depends on the type of explant and specificity of the objectives such as callus medium, shoot proliferation, root formation etc.

The basic concepts of plant tissue culture media are given as (Table 2):

Table 2: Basic Concepts of Plant Tissue Culture Media

Macronutrients	
Nitrogen (N)	Ammonium nitrate (NH_4NO_3) or potassium nitrate (KNO_3).
Phosphorus (P)	Monobasic potassium phosphate (KH_2PO_4).
Potassium (K)	Often included as potassium nitrate (KNO_3) or potassium chloride (KCl).
Calcium (Ca)	Calcium chloride ($CaCl_2$) or calcium nitrate ($Ca(NO_3)_2$).
Magnesium (Mg)	Magnesium sulfate ($MgSO_4$).
Sulfur (S)	Sulfate ions from compounds like magnesium sulfate.

Micronutrients	
Iron (Fe)	Ferric salts or in a chelated form such as Fe-EDTA.
Manganese (Mn)	Manganese sulfate ($MnSO_4$).
Zinc (Zn)	Zinc sulfate ($ZnSO_4$).
Copper (Cu)	Supplied as copper sulfate ($CuSO_4$).
Boron (B)	Boric acid (H_3BO_3).
Molybdenum(Mo)	Sodium molybdate (Na_2MoO_4).
Cobalt	Cobalt chloride(CoCl2)
Iodine	Potassium iodide (KI)
Iron (Fe)	Ferric salts or in a chelated form such as Fe-EDTA.
Manganese (Mn)	Manganese sulfate ($MnSO_4$).
Vitamins and Organic Supplements	
Thiamine HCl (Vitamin B1)	Essential for many cellular processes.
Pyridoxine HCl (Vitamin B6)	Important for amino acid metabolism.
Nicotinic acid HCl (Niacin)	Plays a role in energy metabolism.
Glycine	To promote the plant growth and nutrient uptake.
Myo-inositol/meso-inositol	Often included for cell wall formation and growth.
Carbon Source	
Sucrose	The most common carbon source, provides energy for plant tissue growth.
Glucose or Fructose	Sometimes used as alternative carbon sources.
Gelling Agents (for solid media)	
Agar	The most commonly used gelling agent.
Gelrite	An alternative to agar, providing a clearer medium.
Plant Growth Regulators (PGRs)	
Auxins	Such as indole-3-acetic acid (IAA), naphthaleneacetic acid (NAA), or indole-3-butyric

	acid (IBA), 2,4-Dichloro phenoxy acetic acid (2, 4-D) promote root initiation and callus formation.
Cytokinins	Such as benzylaminopurine (BAP) or kinetin, ziatine, and 2-Dimethylallylamino Purine (2-iP) accelerate shoot proliferation.
***Gibberellins* (GA)**	Used to promote stem elongation and overcome dormancy.
Abscisic acid (ABA)	Often used for the maturation of somatic embryogenesis.
Organic Supplements	
Amino acids	Glutamine or casein hydrolysate can be added to improve growth.
Peptone	Sometimes added as a source of organic nitrogen.
pH Adjustment: The pH of the medium is usually adjusted to around 5.7 to 5.8 before autoclaving.	

Types of Tissue Culture Nutrient Media

Several media formulations are commonly used for the majority of cells and tissue culture work. The four widely used tissue culture media are given in **Table 3.**

- **White's Medium:** This medium was constituted by P. R. White (1963) for the establishment of root cultures of tomato (*Solanum lycopersicum*, Family: *Solanaceae*). It has lower concentrations of salts, including nitrate but a higher concentration of $MgSO_4$.
- **MS Medium:** This medium was established by *Murashige* and *Skoog* (1962) for *in vitro* callus culture of tobacco (*Nicotiana tobacum*, Family: *Solanaceae*). This is the most widely used nutrient medium in plant tissue culture for both dicots and monocots. It has a high amount of nitrogen in the form of nitrate and ammonium having a moderately high ratio of ammonium to nitrate. Its modifications, e.g., half-strength MS (½MS) or quarter-strength (1/4MS) are also commonly used media.
- **B5 Medium:** This medium was developed by O. L. Gamborg (1968) for callus and cell suspension culture of soybean (*Glycine max*, Family Fabaceae). This medium has a lower concentration of ammonium, although it has been widely used for *in vitro* cell, tissue and organ culture in the past.
- **Woody Plant Medium:** This medium was developed by G. Lloyd and B.

H. McCown in 1981 (WPM) for the shoot tip culture of Mountain Laurel (*Kalmia latifolia*, Family: *Ericaceae*). It has then been recognized as a standard medium for propagation of many woody plant species. Potassium nitrate (KNO_3) has been removed from this medium and replaced with potassium sulphate (K_2SO_4).

Table 3: Nutritional composition of four commonly used Plant Tissue Culture Basal Media (White, MS, B5 and WPM):

Macronutrients (mg L^{-1})	**White (1963)**	**MS (1962)**	**B5 (1968)**	**WPM (1981)**
KNO_3	80	1900	2527.5	–
K_2SO_4	–	–	–	990
KH_2PO_4	–	–	–	–
NH_4NO_3	–	1650	–	400
$Ca(NO_3)_2.4H_2O$	–	–	–	556
$Ca(NO_3)_2.2H_2O$	300	–	–	–
$NH_4H_2PO_4$	–	–	–	–
$NaH_2PO_4.H_2O$	19	–	150	–
$(NH_4)_2SO_4$	–	–	134	–
$MgSO_4.7H_2O$	750	370	246.5	370
Na_2SO_4	200	–	–	–
KH_2PO_4	–	170	–	170
$CaCl_2.2H_2O$	–	440	150	96
KCl	65		–	–
Micronutrients (mg L^{-1})				
H_3BO_3	1.5	6.2	3.0	6.2
KI	0.75	0.83	0.75	–
$MnSO_4.4H_2O$	5.0	22.3	–	–
$MnSO_4.H_2O$	–	–	10.0	22.3
$ZnSO_4.7H_2O$	3.0	8.6	2.0	8.6
$Zn(NO_3)_2.6H_2O$	–	–	–	–
$CuSO_4.5H_2O$	0.01	0.025	0.025	0.25

$Na_2MoO_4.2H_2O$	–	0.25	0.25	0.25
$CoCl_2.6H_2O$	–	0.025	0.025	–
$NiSO_4.6H_2O$	–	–	–	–
$FeSO_4.7H_2O$	–	27.8		27.8
Na_2EDTA	–	–	–	37.3
$Na_2EDTA.2H_2O$	–	37.3	–	–
$Fe_2(SO_4)_3$	2.5	–	–	–
Sequestrene330Fe	–	–	28.0	–
Vitamins and Organics (mg L^{-1})				
Myo-Inositol	–	100	100	100
Nicotinic acid	0.05	0.5	1.0	0.5
Pyridoxine HCl	0.01	0.5	1.0	–
Thiamine HCl	0.01	0.1	10.0	1.6
Glycine	3.0	2.0	–	–
Sucrose (g L^{-1})	20	30	20	20
pH	5.5	5.8	5.5	5.6

Source: White (1963), MS: *Murashige and Skoog* (1962), B5: Gamborg et al. (1968), WPM: Woody Plant Medium; Lloyd and McCown (1981)

ACTIVITIES

Activity-1

Preparation of MS (*Murashige & Skoog*) Culture Medium for Growth Promotion

Material Required

- Pen
- Pencil
- Notebook
- *Murashige & Skoog* (MS) basal salt mixture
- Sucrose, *Phytohormones* (e.g., auxins, cytokinins)
- *Agar*
- Distilled water
- pH meter or pH paper

- Autoclave, Glassware (e.g., beakers, flasks)
- Stirrer

Procedure

- Prepare MS basal salt mixture by dissolving the appropriate amount in distilled water.
- Add sucrose to the dissolved MS basal salt mixture and ensure complete dissolution.
- Adjust the pH of the medium to around 5.7 using dilute solutions of hydrochloric acid (HCl) or sodium hydroxide (NaOH).
- Incorporate the desired phytohormones into the medium and mix thoroughly.
- Optionally, add agar to the medium at a concentration of around 0.8-1.0% for solidification.
- Transfer the prepared medium into sterile containers such as culture bottles or petri dishes.
- Autoclave the filled containers at 121°C and 15 psi for 15-20 minutes to sterilize the medium.
- Allow the autoclaved medium to cool down to room temperature before further use.
- Maintain accurate records of the ingredients used, concentrations, pH adjustments, and sterilization process.

CHECK YOUR PROGRESS

A. Fill in the Blanks

1. _____ medium, developed by *Murashige and Skoog*, is used for both dicots and monocots in plant tissue culture.
2. _____ medium, developed by Lloyd and McCown, is standard medium for propagation of many woody plant species.
3. White's medium was formulated for the establishment of root cultures of _____
4. B5 medium was developed for callus and cell suspension culture of _____.

B. Multiple Choice Questions

1. Which tissue culture media is known for its higher concentration of $MgSO_4$?

   a) White's medium

b) MS medium
c) B5 medium
d) Woody Plant Medium

2. What is the main function of auxins in tissue culture?
   a) Promote root initiation
   b) Induce callusing
   c) Stimulate shoot elongation
   d) All of the above
3. Which of the following is a natural *auxin*?
   a) 2,4-D
   b) NAA
   c) IBA
   d) IAA
4. What is the primary role of cytokinins in tissue culture?
   a) Inhibit root formation
   b) Stimulate shoot multiplication
   c) Induce callus formation
   d) Induce flowering

5. Which gelling agent is derived from the bacteria Pseudomonas elodea?
   a) Agar
   b) Agarose
   c) Gellan gum
   d) PGR

C. Subjective Questions

1. Describe the various types of tissue culture nutrient medium.
2. Describe the organic nutrients of media constituents.
3. Explain the inorganic nutrients of media constituents.
4. Describe the different gelling agents.

Session 2: General Methodology of Media Preparation

Media preparation is one of the primary and most critical steps in plant tissue culture where precision is required. There are two methods of preparing medium:

(i) The first is to weigh and dissolve the necessary elements individually before mixing them. However, this procedure is more time-consuming and prone to errors.

(ii) The second more convenient and widely practised method is to prepare, a series of concentrated stock solutions (i.e. 50x, 100x, 200x, where x= time concentrated solution) in advance and store them in a refrigerator. To prepare a medium, draw the necessary amounts from the stock solutions and mix them.

Preparation of stock solution

Preparation of a Stock Solution refers to the process of making a concentrated solution from which more diluted solutions can be prepared for experimental use. Stock solutions are often used in laboratory settings to simplify the process of making multiple solutions with varying concentrations.

Preparation of NH_4N0_3 (50x) stock solution

- Requirement of NH_4N0_3 in ms media is – 1650mg/litre or 1.65gm/litre
- NH_4N0_3 (50x) = 1.65×50 = 82.5gm
- To prepare stock 82.5gm of NH_4N0_3 weigh and dissolved in 1000ml of distilled water

Use of stock

- 82.5gm NH_4N0_3 available in 1000ml.
- 1.65gm NH_4N0_3 will be available in $= \frac{1.65\times1000}{82.5}$ = 20ml
- Similar calculations can be used also for 100x, and 200x.

Table 4: Composition of Stock Solutions for* Murashige and Skoog *(MS, 1962) medium:

Stocks	Constituents	Final concentration (MS Media) (mg l^{-1})	Stock Strength	Concentrations of stock solution (g l^{-1})	Final Vol. to be taken for 1 liter MS media (ML)
A	NH_4NO_3	1650.00	50X	82.5	20
B	KNO_3	1900.00	50X	95.0	20
C	H_3BO_3	6.20	200X	01.24	5
	KH_2PO_4	170.00		34.0	
	KI	0.83		00.166	
	$Na_2MoO_4.2H_2O$	0.25		00.05	
	$CoCl_2.6H_20$	0.025		00.005	
D	$CaCl_2.2H_2O$	440.00	200 X	88.0	5
E	$MgSO_4.7H_2O$	370.00	200X	74.0	5
	$MnSO_4.7H_2O$	22.30		04.46	
	$ZnSO_4.7H_2O$	8.60		01.72	
	$CuSO_4.5H_2O$	0.025		00.005	
F	$Na_2EDTA.2H_2O$	37.30	200X	07.46	5
	$FeSO_4.7H_2O$	27.80		05.56	
G	Glycine	2.00	100X	00.2	10
	Thiamine HCl	0.10		00.01	
	Nicotinic acid	0.50		00.05	
	Pyridoxine HCl	0.50		00.05	
	Meso-inositol	100.00		Need not to prepare stock solution	At directly at the time of media preparation
	Sugar	30000.00			
	Agar-agar	8000.00			

*Adjust the pH of the medium to 5.8 ± 0.02

5.1 Process of Stock Solution Preparation

For preparation of stock, initially dissolve each salt completely before mixing with the others. To prevent precipitation, group chemicals by their ions (e.g. nitrate, sulphate, phosphate, and halide) and mix the dissolved components of each group before mixing the entire solution. Stock solutions of macro and micro salts are stored separately. To prepare the iron stock solution (Stock F), weigh and dissolve $FeSO_4.7H_2O$ and $NA_2EDTA.2H_2O$ separately in 450 ml of distilled water by heating and continuous stirring, then mix them. Distilled water is added to make up the final volume to one litre and the pH is adjusted to 5.5 (Stock F). This stock solution should be a deep golden yellow and must be stored in an amber bottle.

Similarly, stock solutions for PGRs can be made at 1- or 10-mM strength, depending on their amount of use. To dissolve the PGRs, a small amount of solvent is used, followed by a final volume made up of distilled water. To prepare stock solutions and media, utilize purified water (double distilled/reverse osmosis) and high-purity chemicals.

Precautions

- All stock solutions should be kept refrigerated in plastic or glass bottles.
- Label (Figure 3.1) all bottles containing stock solutions with the following information: medium type, stock type, amount to be taken, preparation date and investigator's name. Before using the stocks, gently shake the bottles.

Fig: 7 Bottle Labels

5.2 Steps for Preparing Culture Medium

- Take 100ml distilled water in 1000ml of beaker.
- Add appropriate stock solutions, including PGRs and other specific supplements. Add thermolabile substances that may be filter sterilized through microfilters (pore size 0.22–0.45 µm) and added to the autoclaved medium under a Laminar Air Flow Hood.
- The final volume of medium is made up of 980ml using distilled water. After mixing it with the media constituents, the pH of the

medium is generally adjusted to 5.8±0.01 with 0.1 N NaOH or 0.1 N HCl.

- Finally, make up the volume 1000ml with the help of distilled water.
- At last add 0.8% *Agar* boil it and completely dissolve the agar with continuous shaking.
- Cool down the medium at 45C and Pour into the suitable culture containers. Dispense about 20-25 ml of the medium in a 150 ml Erlenmeyer flask.
- Plug the culture vessels with non-absorbent cotton wrapped in muslin cloth to prevent microbial contamination while allowing gaseous exchange.
- Medium-containing culture vessels are autoclaved at 1.06 kg cm-2 (15 psi) for 15 minutes (duration varies depending upon medium volume).
- After cooling, the medium is stored at room temperature. The nutrient medium should preferably be used within a week.

Precautions

- To minimize human errors, carefully follow the instructions given above. To keep track of the medium's ingredients, list them on paper and cancel them individually when they are added.
- Mark/label clearly all culture vessels containing the medium so they can be identified even after autoclaving and storage under light.

5.3 Media Preparation Using Pre-Mixed Sachet

Nowadays, a wide range of basal and special media formulations are available commercially for sale in the form of pre-mixed dry powder and packaged in sachets of different quantities. To prepare the medium, dissolve the powder in distilled water (10% less than the final volume), then add sucrose, agar, and any other required supplements; and make the final volume with 980ml of distilled water. Adjust the pH of the medium and finally make up the volume 100ml with the help of distilled water. Add 0.8% of Agar before autoclaving it. These powdered media are more effective for commercial micropropagation of specific crops. It saves time by eliminating the need to prepare, store and mix multiple chemicals.

6. Media for different Tissue Culture Purposes

6.1 Multiplication Medium

Nutrient medium formulation for the multiplication phase is important. The media prepared for culture initiation is the same and can be used as a multiplication medium by just amending the PGRs (preferably by

lowering their concentrations). In general, *cytokinins* alone or in combinations are incorporated in the medium for shoot multiplication. However, it has been reported that the addition of *auxin* to this media enhances the shoot multiplication. It is well documented that combinations of *cytokinins*-auxins act synergistically and thus, improve the multiplication rate.

6.2 Rooting Medium

For rooting, the basic steps of media preparation are the same as those of multiplication media. However, several differences should be carefully followed:

- Generally, the strength of basal nutrient media is reduced up to one-half or one-fourth, e.g., MS1/2, MS1/4.
- For this, the volume of stock solutions (A to G) taken for media preparation is reduced to half or one-fourth. The concentration of sugar is also reduced up to 2% (20 g L^{-1}).
- *Auxins* (IBA, NAA, IAA) are incorporated in the rooting medium after standardization, as per the requirement of the species.
- Other parameters are kept the same as for the multiplication media.

7. Calculation of Normality and Molarity

7.1 Normality

Normality (N) is defined as the number of grams or equivalent weight of solute contained in one litre of a solution. Equivalent weight is the mass of the substance in grams required to react with or replace one gram of hydrogen. In other words, it is defined as the molecular mass of a substance divided by the number of replaceable H+ or OH- ions, valency, or number of electrons.

Normality is expressed as:

$$\text{Normality (N)} = \frac{\text{equivalents weight (gm)}}{Volume\ (1000\ ml)}$$

Equivalent weight is expressed as:

$$\text{Equivalent weight (E)} = \frac{\text{Mol. Wt. of substance}}{n}$$

Where n is the number of replaceable H+ ions (for acids) or OH- ions (for bases)

7.2 Molarity

It is defined as the number of moles of solute in one litre of a solution. It is also called as molar concentration and expressed as:

$$\text{Molarity (M)} = \frac{\text{molecular weight (gm)}}{Volume\ (1000ml)}$$

In plant tissue culture, the concentration of PGRs is often expressed in micromolar (µM). To convert micromolar concentrations to mg L-1, the following formula can be used:

$$\text{Conc. (mg L}^{-1}) = \frac{\text{Conc.(µM)} \times \text{Mol.Wt.}}{1000}$$

Activities

Activity-1

Prepare a Solution of Given Strength (Normality & Molarity, Parts per Million).

Material Required:

- Pen
- Pencil
- Notebook
- Chemical reagents (e.g., NaCl, HCl, NaOH)
- Distilled water
- Balance
- Volumetric flasks
- Beakers
- Stirring rod

Procedure

- Choose the chemical needed for the solution, ensuring its purity is suitable for the intended purpose.
- Calculate the required amount of the chemical based on the desired concentration and volume of the solution using appropriate formulas.
- Accurately measure the calculated amount of the chemical using a balance, while wearing safety goggles and gloves.
- Dissolve the measured chemical in a suitable volume of distilled water in a clean and dry container for molarity and normality solutions. For ppm solutions (mg per litre), dissolve the chemical in a known volume of water to achieve the desired concentration.
- Mix the solution thoroughly by stirring or swirling until the chemical is completely dissolved.

- Adjust the volume of the solution if necessary by adding distilled water carefully to achieve the desired final volume, using a graduated cylinder or volumetric flask for precise measurements.
- Clearly label the container with the name of the chemical, concentration, date of preparation, and any relevant safety information.
- Dispose of any leftover chemicals or waste solutions according to laboratory safety guidelines and regulations.
- Clean all equipment used in the preparation process thoroughly to prevent contamination in future experiments.
- Record all details of the solution preparation, including calculations, measurements, and any observations made during the process, in a laboratory notebook or log.

CHECK YOUR PROGRESS

A. Fill in the Blanks

1. Stock solutions should be kept refrigerated in _____ bottles.
2. Pre-mixed dry powder media formulations are available commercially in _____
3. Multiplication medium often contains _____ for shoot multiplication.
4. Rooting medium may include activated charcoal and specific _____ for rooting.
5. Normality is expressed as the number of gram equivalents per _____.

B. Multiple Choice Questions

1. How are chemicals grouped during the process of stock solution preparation?
   a) By their colours
   b) By their molecular weights
   c) By their ions
   d) Randomly
2. What is used to adjust the pH of the medium?
   a) Distilled water
   b) Agar
   c) NaOH or HCl

d) Sucrose

3. What is added to prevent microbial contamination in culture vessels?

   a) Non-absorbent cotton
   b) *Agar*
   c) Sucrose
   d) Activated charcoal

4. What is the purpose of adding activated charcoal in rooting medium?

   a) To enhance shoot multiplication
   b) To prevent microbial contamination
   c) To absorb toxins
   d) To improve root development

5. What is normality defined as?

   a) Number of moles of solute in one litre of solution
   b) Number of gram equivalents per litre of solution
   c) Mass of the substance in gram required to react with one gram of hydrogen
   d) Number of moles of solute divided by volume of solution

C. Subjective Questions

1. What are the advantages and disadvantages of using pre-mixed sachets for tissue culture medium?
2. What is the importance of proper labelling and storing of stock solutions in plant tissue culture?
3. What precautions should be taken for preparing and autoclaving culture medium to maintain sterility?
4. What are the methods to calculate the normality of a solution with 0.5g HCl dissolved in 100ml water?

Session 3: Preparation of Buffers and Solutions

Everything you know operates at a particular pH. Even our bodies' cells, the water we consume, and the plant in your garden have their preferred pH values. Similarly, in plant tissue culture, where plants grow in a controlled environment on a nutrient-rich medium, they require a precise pH of the medium. The solubility of salts, the effectiveness of agar-gelling, and the uptake of nutrients by tissues in culture are significantly influenced by the pH of the nutrient medium.

A pH meter is required to prepare the media of desired pH. The pH meter consists of three parts: (i) a reference electrode (ii) an internal electrode, and (iii) a high-input impedance meter. The glass probe that measures the pH consists of a reference electrode and an internal electrode [contains a silver wire (Ag) covered with Silver Chloride (AgCl wire)]. In the glass probe, there is a buffer solution of pH 7. The pH is measured as the difference in $[H^+]$ between the reference buffer (inside the glass probe) and the sample solution outside.

Calibration of the pH Meter

Before measuring the pH of any solution, calibration of the pH meter with appropriate buffer solutions is essential. It is required to correct variations or errors in pH measurements and should be done before each use.

Calibration Procedure:

- Turn on the pH meter and wait for about 30 minutes for the machine to warm up.
- Remove the electrode from the storage solution, rinse it with distilled water, and wipe using tissue paper (do not rub the electrode).
- Prepare the buffer solutions (using buffer tablets). For calibrating a pH metre, three types of buffers (pH 4, pH 7 and pH 10) are required. Allow the buffers to reach the same temperature as the pH metre, because pH values are temperature-dependent.
- Put the electrode in the pH 7 buffer and push the measure or calibrate button to start reading the pH.
- Wait up to 1 minute to allow the pH reading to stabilize.
- Once we get a stable reading, press the measure button again to calibrate the pH metre to the buffer's pH. When the pH metre is calibrated, it produces more specific results.
- Rinse the electrode with distilled water and wipe.
- Repeat the preceding steps using buffers with pH 4 and pH 10. For regular use, calibration using two buffers is sufficient.

Measurement and Adjustment of pH

The pH is generally adjusted to 5.5 to 5.8 (slightly acidic and depends on the media type) in plant tissue culture to keep all salts in a near-buffering state. However, it is also plant species-specific, as some plants grow and develop better in slightly acidic or basic pH media. It has been observed that a pH above 6.0 results in a hard medium, while a pH below 5.0 prevents agar from gelling appropriately due to hydrolysis during autoclaving. The pH of culture media decreases by 0.1-0.3 units after autoclaving and fluctuates during the culture period due to oxidation, ion absorption, and tissue secretions. It has been reported that an organic buffering agent, 2-(N-morpholino) Ethane Sulphonic Acid (MES), is incorporated into the medium after adjusting pH to 5.7 for maintaining a buffered medium for longer incubation intervals.

The procedure of pH Measurement

The pH is measured after mixing all the constituents of media including PGRs and making the final volume using distilled water on a magnetic stirrer before autoclave:

- Rinse the electrode in distilled water and wipe it with tissue paper.
- Adjust the temperature of the pH meter to that of the medium.
- Place the electrode in the media and press the measure button.
- The pH value will be displayed on the meter's display screen.
- Allow 30 seconds for the pH to stabilise.
- If the pH is higher (basic) than desired, apply 1N HCl to lower it and adjust to the desired level.
- If the pH is lower (acidic), then add 1N NaOH to increase and adjust it to the desired level.

Precautions: Before placing the electrode in any solution to measure the pH, make sure it is rinsed, cleaned, and pat dried.

Various types of Plant Tissue Culture Assays

Conducting various types of assays in plant tissue culture provides valuable insights into different aspects of plant cell behaviour. These assays contribute to the development of resilient crops, efficient propagation methods, and applications in biotechnology and agriculture.

a. Proliferation Assay

A proliferation assay in plant tissue culture is a method used to assess the rate of cell division and the overall growth and multiplication of plant cells *in vitro*. This assay is particularly important in plant tissue culture research, where scientists aim to propagate and maintain plant cells or tissues under

controlled conditions. The rate of proliferation can be assessed by measuring various parameters, which may include:

- **Growth Rate:** Measure the increase in size or fresh weight of the cultured tissues over time.
- **Cell Counting:** Use an automated cell counter to quantify the number of cells in each sample.
- **Dry Weight Analysis:** Determine the dry weight of the tissue as an indicator of biomass accumulation.

By conducting proliferation assays in plant tissue culture, researchers can gain insights into the growth dynamics of plant cells, optimize culture conditions, and develop strategies for large-scale propagation and regeneration of plants in a controlled environment.

b. Apoptosis Assay

Apoptosis is a regulated and controlled process that occurs in plant cells as part of normal development, stress responses, and defence mechanisms. Detecting and quantifying apoptosis in plant tissue culture is important for understanding cellular responses to various stimuli or environmental conditions. For visualizing apoptotic cells, various staining techniques can be utilized. The common stains used for detecting apoptosis in plant cells include:

- **Fluorescent Dyes:** Dyes such as acridine orange or propidium iodide can be visualized under a fluorescence microscope. These dyes can highlight apoptotic features, such as nuclear condensation and fragmentation.
- **Terminal deoxynucleotidyl transferase dUTP Nick End Labelling (TUNEL) Assay:** This technique labels the 3'-OH ends of fragmented DNA, providing a specific marker for apoptotic cells.

By conducting apoptosis assays in plant tissue culture, researchers can gain insights into the regulatory mechanisms of programmed cell death, understand how plants respond to various stresses, and potentially develop strategies for enhancing stress tolerance in crops.

Activity

Demonstrate the pH measurement of a given solution.

Material Required

- Pen
- Pencil
- Notebook

- Distilled water
- Electronic balance
- Volumetric flasks
- Beakers
- Stirring rod

Procedure

1. Identify different chemicals and glassware used in pH measurement.
2. Measurement of required chemicals.
3. Prepare a solution of a given strength
4. Precautions are taken during the preparation of the solution and measurement of pH.

A. Fill in the Blanks

1. The glass probe of a pH meter consists of a reference electrode and an internal electrode containing a silver wire covered with _____.
2. Calibration of a pH meter involves using buffer solutions with pH values of _____, _____, and _____.
3. The pH of plant tissue culture medium is generally adjusted to _____ to _____.
4. pH values in plant tissue culture media tend to decrease by _____ units after autoclaving.
5. An organic buffering agent like MES is incorporated into the medium to maintain a _____ medium for longer incubation intervals.

B. Multiple Choice Questions

1. What is the purpose of calibrating a pH meter with buffer solutions?

   a) To measure the temperature of the solution
   b) To correct errors in pH measurements
   c) To adjust the internal electrodes
   d) To determine the concentration of ions in the solution

2. Why is it important to allow buffer solutions and the pH meter to reach the same temperature?

   a) To maintain consistency in pH readings
   b) To prevent the buffer solutions from evaporating
   c) To speed up the calibration process
   d) To reduce the risk of contamination

3. Which pH range is generally preferred for plant tissue culture medium?

   a) 6.0 to 7.0
   b) to 5.0
   c) to 5.8
   d) 7.0 to 8.0

4. What is the purpose of incorporating an organic buffering agent like MES into the medium?

   a) To increase the pH stability
   b) To decrease the pH after autoclaving
   c) To prevent agar from gelling
   d) To promote tissue secretion

5. What should be done if the pH of the medium is higher (basic) than desired?

   a) Add 1N NaOH to increase it
   b) Apply 1N HCl to lower it
   c) Adjust the temperature of the medium
   d) Rinse the electrode with distilled water

C. Subjective Questions

1. Explain the significance of pH calibration in plant tissue culture.
2. How does pH affect the solubility of salts and the effectiveness of agar-gelling?
3. What are the steps involved in adjusting the pH of the medium using acid or base solutions?
4. Describe the role of apoptosis assays in plant tissue culture research.

Compare and contrast the methods used for assessing cell proliferation and apoptosis in plant tissue culture.

Module 4

Maintenance of Aseptic Condition in a Plant Tissue Culture Laboratory

Module Overview

Learning outcomes

After completing this module, you will be able to:

1. Understand the process of sterilizing and properly storing the culture medium to prevent contamination.
2. Learn essential biosafety practices and cleanliness measures required in a plant tissue culture laboratory.

Module structure

Session 1: Process of Sterilization and Storing the Culture Medium

Session 2: Biosafety Practices and Cleanliness in a Plant Tissue Culture Laboratory

Session 1: Process of Sterilization and Storing the Culture Medium

Sterilization

Sterilization is an important step in plant tissue culture to prevent contamination and ensure the successful growth of plant tissues in vitro. Sterilization is critical for the success of plant tissue culture, ensuring that explants remain uncontaminated throughout the culture process, thereby maximizing the chances of successful plant growth and development.

It is performed to preserve items for a long time and eliminate various germs such as bacteria, viruses, fungi, and other microorganisms. Sterilization aims to maintain sterility, which requires sufficient time and efficiently eradicates microbes. This process is particularly important for instruments, surgical gloves, and other glassware. The sterilization depends on the quantity and type of microorganisms' present.

In plant tissue culture, microbial contamination (bacterial/fungal) is a major problem and causes serious economic loss. Contamination can occur from the laboratory environment, instruments, lab ware, culture medium, plant material, or even the operator. Therefore, maintaining an aseptic environment inside a plant tissue culture laboratory is essential and critical. The process of removing/killing all forms of microbes from the nutrient media, explant or any object/instrument/equipment involved in the *in vitro* operations are called Sterilization.

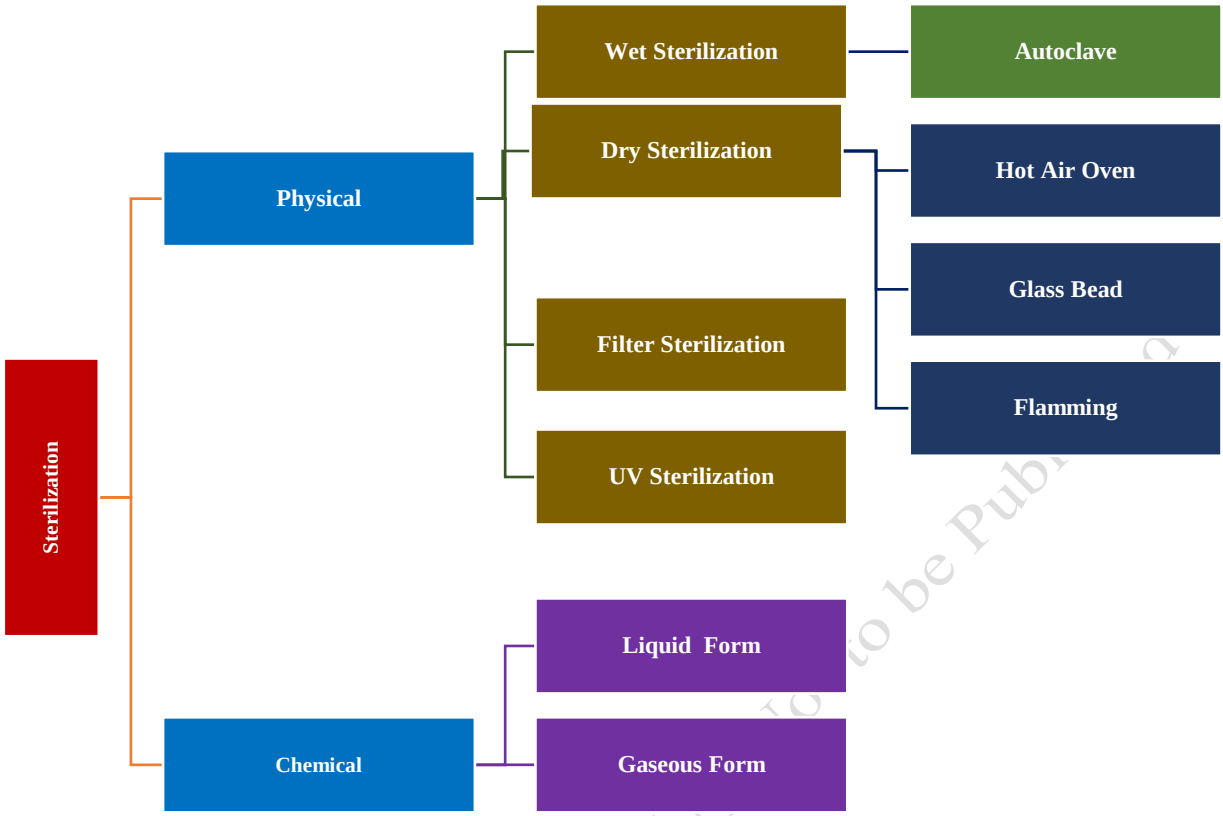

Fig: 8 Type of sterilization techniques

Sterilization Techniques Useful in Plant Tissue Culture

The common sterilization techniques used in plant tissue culture are as follows:

1. Surface Sterilization of Explants:

A. Chemical Sterilization:

- **Sodium Hypochlorite (NaOCl):** A common disinfectant, often used in a 0.5% to 1.0% solution for 5-15 minutes. It is effective against bacteria and fungi.
- **Mercuric Chloride ($HgCl_2$):** It is a potent sterilizing agent typically used at 0.1% to 0.2% concentration for 2-10 minutes. It is highly effective but toxic, requiring careful handling and disposal.
- **Ethanol (70%):** It is used for short durations (30 seconds to 1 minute) before other sterilization steps to quickly kill surface contaminants.
- **Hydrogen Peroxide (H_2O_2):** Used at concentrations of 3% to 10% for 5-15 minutes, it is effective in removing contaminants without damaging plant tissues.

Table 5: Various chemicals used in sterilization of explant

Chemical sterilant	Working concentration	Effectiveness	Treatment time (Min)	Remarks
Sodium hypochlorite	1-1.4 %	+++	5-30	Very effective
Calcium hypochlorite	9-10 %	+++	5-30	Very effective
Hydrogen peroxide	10-12 %	+++++	5-15	Effective
Bromine water	1-2 %	+++	2-10	Very effective
Silver nitrate	1 %	+	5-30	Effective
Mercuric chloride	0.01-1 %	+	2-10	Satisfactory
Antibiotics	4.50 mg/l	++	35-55	Effective

B. Physical Sterilization:

- **Autoclaving:** It is utilized for sterilizing culture media, tools, and containers by subjecting them to high-pressure steam at 121°C for 15-20 minutes. Autoclaving is effective in killing all forms of microorganisms.
- **Dry heat sterilization**: Dry heat is the process of sterilizing objects or materials using dry heat, typically in a hot air oven. Fungal spores are killed at 115°C within 60 minutes, and bacterial spores are killed at 120-160°C within 60 minutes. Glass, culture vessels, metal instruments, and aluminium foil can be sterilized by exposure to dry air (160-180°C) for 2-4 hours in a hot air oven. There are two methods of dry heat sterilization:
    i. **Hot Air Oven:** This is a commonly used method of sterilization of Glassware, swab sticks, all-glass syringes, powders, and oily substances. Sterilization is achieved by maintaining a temperature of 160°C for one hour, effectively killing spores and ensuring sterility.

ii. **Glass Bead Sterilizer:** Glass bead sterilizers are the most effective for sterilizing metallic tools. These sterilizers heat to around 275 to 350 °C, destroying bacterial or fungus spores. The handling metallic tools (forceps, scissors, scalpel, spatula, needle) are simply put into the heated glass beads for up to one minute (Fig. 15). Then, keep these items on a rack under the Laminar air flow hood to cool before using it or the same can be cool down by a dip in alcohol.

- **UV Sterilization:** UV light can be used to sterilize the air and surfaces in the culture room, reducing airborne contamination. Irradiation is a method of sterilization that involves exposing surfaces or objects to different types of radiation such as gamma rays, X-rays, and ultraviolet (UV) light. These radiations target microbial DNA, causing damage through processes like ionization and free radical production. Ultraviolet (UV) radiation, a non-ionizing form of radiation, is particularly effective for sterilization. It includes light rays ranging from 150 to 3900 angstroms, with 2600 angstroms being the most bactericidal. UV radiation is commonly used to sterilize air, sanitize work areas, and treat water used in tissue culture processes. Ionizing radiation, such as X-rays and gamma rays, is also utilized for sterilization. When exposed to ionizing radiation, water molecules undergo ionization, producing toxic oxygen metabolites like hydroxyl radicals, superoxide ions, and hydrogen peroxide.

2. Sterilization of Culture Media

The sterilization of culture media is a crucial and necessary step in plant tissue culture. Bacteria and fungi are frequent contaminants found in tissue cultures. Fungal spores are lightweight and found throughout the environment; when they come into contact with media, conditions are favourable for their luxuriant growth leading to contamination. Nutrient media can be sterilized by steam sterilization (autoclaving) and membrane filtration.

- **Autoclaving:** As mentioned, media are commonly sterilized in an autoclave at 15psi pressure, 121°C for 15-20 minutes to kill any microorganisms present. An autoclave (also called steam sterilizers) is a machine that utilizes steam and pressure to sterilize objects by destroying microbes (bacteria, viruses, fungi) and their spores. An autoclave works on the principle of a pressure cooker used in a kitchen. Autoclaves can be vertical (Fig. 18) or horizontal (Fig. 17) and are of various sizes and capacities. Vertical autoclaves are difficult to operate above a certain limit due to their depth (crucial while loading and

unloading). Horizontal autoclaves are easier to use but are costlier to purchase and expensive to operate (electricity charges). Horizontal autoclaves can have either a single or double-door system. The double-door autoclaves are installed in such a way that one door opens in the media room to load the media, while the other door opens directly into the 'clean area' to unload the sterilized media.

- **Filtration:** For heat-sensitive components like growth hormones (e.g., *auxins*, *cytokinins*), sterile filtration using a 0.22 µm filter is employed to remove contaminants without exposing the media to high temperatures. Filter sterilization is required for thermolabile substances like some PGRs (Zeatin, GA3), carbohydrates, vitamins, amino acids, and plant extracts, as they get destroyed/lost their efficiency while autoclaving. To filter-sterilize a solution, bacteria-proof membranes (made of cellulose acetate or cellulose nitrate) with pore sizes of 0.22-0.45 microns (µm) are used. During membrane filtration, all microorganisms and particles larger than the pore diameter are eliminated. To sterilize the filter membrane, place it in the proper size filter holder and wrap it in aluminum foil while autoclaving. Before membrane filtration, the empty glass/plastic ware used to pour the media must be autoclaved. The sterilized liquid drops (of the thermolabile compound) are directly poured into the autoclaved medium cooled to 40-50°C in case of semi-solid medium and at room temperature in case of liquid medium under aseptic conditions. Membrane filtration for large volumes can be done with a vacuum pump attached to a filtration setup.

3. Sterilization of Instruments and Equipment

- **Flaming:** Instruments such as forceps and scalpels are often sterilized by dipping them in ethanol and then flaming them before use. Metallic tools (forceps, scissors, scalpel, spatula, needle) can also be sterilized by dipping them in ethanol (70%) followed by flaming on a burner (spirit lamp) and then cooling down before their use. However, as the ethanol is highly volatile and inflammable, this step carries the risk of fire. Therefore, extra precautions should be taken while performing this sterilization. To detect any fire, the lights of the Laminar Air Flow Bench should be switched off prior to starting this sterilization.
- **Autoclaving:** Instruments and reusable culture vessels are autoclaved to ensure they are free from contaminants. Moist heat sterilization is the process of using pressurized steam to heat the material for sterilization. This is an effective way to kill all microbes, spores, and viruses, but requires particularly high temperatures or specific times

for some organisms. Boiling the method that effectively eliminates all non-sporing organisms within 5-10 minutes. It is commonly used to disinfect items like blades and syringes. However, after removal from the boiling water, an object should be allowed to dry thoroughly before handling to prevent contamination by bacteria from the skin.

4. Aseptic Techniques

- **Laminar Air Flow:** Used to maintain a sterile environment while working with plant tissues. The hood provides a flow of filtered air that helps to prevent contamination from airborne particles.
- **Personal Protective Equipment (PPE):** Wearing gloves, lab coats, and sometimes masks helps to minimize the introduction of contaminants from the operator.

5. Sterilization of Seeds and Plant Material

- **Pre-treatment Washing:** Explants are often pre-washed with running tap water or a mild detergent solution to remove surface debris.
- **Sequential Sterilization:** Involves a combination of different chemical sterilants in sequence to effectively reduce contamination while minimizing damage to the plant material.

Storage of Culture Media

It is observed that certain Bacillus bacteria can withstand autoclaving. Therefore, it is appropriate to incubate the autoclaved nutrient media in the storage room (30-32 °C) for 2 to 3 days before use to verify its proper decontamination. It is important to keep all media away from light. Agar solidified medium should be stored in airtight containers to prevent moisture loss. Media should not be refrigerated. It is observed that fresh media performs better than stored media, thus avoiding extensive storage times is always advised. Selective components of media have a relatively short active life; hence such media should be used within a few days of preparation. Date-stamping containers or culture vials is a good laboratory practice. The prepared media should be checked before inoculation. Look for contamination, uneven filling or bubbles on the media surface, colour changes, and dehydration indicators, such as shrinking/cracking, and loss of volume. Discard the culture vial, if there is any defect.

The procedure of Explant Sterilization

A general explant sterilization procedure requires the following steps:

Washing: The explants need to be washed with running tap water to remove dust or any other external material adhering to the explant**.** Now take a beaker fill it with distilled water and add a few drops of detergent such as Tween 20.

Immerse the explant in this solution and stir continuously. Then, rinse the explant with a few changes of distilled water to remove traces of detergent on the explant**.** The washing of the explant can be done in the laboratory working area, further surface sterilization needs to be carried out on the Laminar Air Flow Bench in the Clean Area.

Chemical Treatment

- The explants are then treated with Alcohol in a beaker or flask for a few seconds and dried on a petri dish to evaporate extra alcohol. Flasks are preferred as stirring is easy in them. All glassware used for surface sterilization procedure should be sterile.
- The explant is treated with surface disinfectant chemicals like Mercuric Chloride or Hypochlorite in the appropriate concentration as mentioned above.
- The explants should be continuously shaken or stirred while treated with surface sterilizing chemicals for effective treatment.

Rinsing

- The explants are rinsed with sterile distilled water 4-5 times so that all traces of chemicals can be removed.
- The surface sterilized explants are kept on Petri plates to be inoculated in a culture medium with the help of sterile forceps.

Special Treatment: In case of heavy contamination, explants can be treated with fungicides and antibiotics. The concentration and time of fungicide and antibiotic treatment depend on the extent of contamination. Generally, 1% solution is used for 5-20 minutes.

Precautions

- Surface sterilization procedure for explants needs expertise as concentration and time of chemical treatment need to be standardized for every plant material. The nature of treatment depends on the hardiness of plant material. Soft materials like leaves need less time and low concentration of chemicals while woody stems need more time and high concentrations as mentioned in the range of concentration of chemicals.
- A general precaution while preparing chemicals should be followed as mentioned on chemical bottles. A correct concentration should be prepared for effective sterilization. The weighing of chemicals should be accurate. Dispensing of liquids should be done with micropipettes or measuring cylinders. Mouth pipetting of chemicals used for surface sterilization should be completely avoided.

- All the chemicals used for surface sterilization of explants are toxic and corrosive. Contact with eyes and hands should be avoided. Accidental touch or ingestion of these chemicals should be treated immediately. First Aid should be administered as mentioned on chemical bottles and immediate medical treatment is advised.
- All chemicals should be stored in conditions mentioned on labels of bottles. They should always be kept in their original bottles and should not be mislabeled

Best Practices for Sterilization Process

- **Sterilization Timing:** Carefully control the duration of exposure to sterilants to avoid tissue damage.
- **Sterilant Concentration:** Use the minimum effective concentration to reduce toxicity while ensuring thorough sterilization.
- **Post-Sterilization Rinsing:** Explants are typically rinsed several times with sterile distilled water to remove any residual sterilants.

ACTIVITIES

Activity-1

Demonstrate the process of sterilization of glassware.

Material Required:

- Glassware (such as beakers, flasks, pipettes)
- Oven
- Distilled water
- Gloves

Procedure

1. Rinse glassware with distilled water to remove debris.
2. Place the glassware in the oven and seal securely.
3. Set the temperature for sterilization as recommended.
4. Monitor the sterilization process closely.
5. Remove glassware with heat-resistant gloves.
6. Allow to cool before use.

CHECK YOUR PROGRESS

A. Fill in the blanks

1. Dry heat sterilization is commonly achieved by maintaining a temperature of _____ for one hour.

2. Autoclaving is typically performed at a pressure of _____ psi and a temperature of 121°C.
3. The most bactericidal wavelength of UV radiation ranges from _____ angstroms.
4. Sodium hypochlorite, commonly known as bleach, is diluted to a final solution of _____% for tissue culture sterilization.
5. Ethanol, employed for explant sterilization, is typically used at a concentration of _____% (v/v).

B. Multiple choice questions

1. What is the most common method for sterilizing glassware in laboratories?

   a) Autoclaving
   b) Flaming
   c) Filtration
   d) Irradiation

2. Which chemical is highly toxic and therefore rarely used in labs for explant sterilization?

   a) Sodium Hypochlorite
   b) Hydrogen Peroxide
   c) Mercuric Chloride
   d) Ethanol

3. What is the concentration of bleach commonly used for sterilizing plant explants?

   a) 1-2%
   b) 5-10%
   c) 10-20%
   d) 30%

4. What is the Temperature of dry heat sterilization?

   a) 100-120°C
   b) 160-180°C
   c) 121°C
   d) 200-220°C

5. What is the purpose of flaming in sterilization?

a) To expose objects to ultraviolet light
b) To expose objects to dry heat
c) To burn microorganisms using a gas flame
d) To pass solutions through a filter

C. Subjective Questions

1. Explain the method of sterilization.
2. Explain the process of sterilizing plant material in tissue culture and its importance in ensuring successful culture growth.

Session 2: Biosafety Practices and Cleanliness in a Plant Tissue Culture Laboratory

Biosafety is the combined use of laboratory practices, laboratory facilities and safety equipment. Used biosafety practices to protect workers/students, products/experimental results and environment/laboratory classroom. Biosafety measures are categorized into different levels:

Biosafety Levels

- **Biosafety Level 1** (BL-1) is the basic level of protection and is appropriate for agents that are not known to cause disease in normal, healthy humans. This level is used in most research and clinical laboratories.
- **Biosafety Level 2 (BL-2)** is used when working with agents that pose a moderate risk to human health. These agents can cause diseases of varying severity when they enter the body through ingestion or contact with the skin or mucous membranes. BL-2 is commonly applied in tissue culture laboratories.
- **Biosafety level 3 (BL-3)** is suitable when working with agents that have known potential for aerosol transmission, agents that may cause serious and potentially lethal infections and that are indigenous or exotic in origin. All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment.
- **Biosafety Level 4 (BL-4)** is the appropriate level when working with Dangerous and exotic agents that pose a high risk of life-threatening disease by infectious aerosols and for which no treatment is available. The agents such as all viruses include the Marburg virus, and Ebola virus.

Good Laboratory Practices Followed in a Plant Tissue Culture Laboratory

In the laboratory, certain protocols must be strictly followed to ensure safety, hygiene, and the integrity of experiments. It is imperative to adhere to the following guidelines:

Consumption of food and beverages and smoking within the laboratory premises are prohibited to maintain a clean and safe environment. Personal Protective Equipment (PPE), including appropriate attire and gear, must be always worn while inside the laboratory to minimize risks associated with experimental procedures. Lab benches must be cleaned, disinfected, or decontaminated after completion of work to prevent cross-contamination. Storage of vessels within laminar airflow is prohibited to prevent obstruction and ensure optimal airflow.

Glassware must be inspected for any signs of damage such as cracks, breaks, or chips before use, and broken glass containers are to be properly disposed of, with only intact glassware utilized. Heavy objects should be stored on lower shelves to prevent accidents and ensure stability, while regular cleaning of the tissue culture laboratory using disinfectants minimizes microbial contamination. Strict attention to detail during media preparation, including precise measurement, thorough mixing, proper sterilization, and aseptic transfer within a laminar airflow hood, is crucial. Additionally, sterilization of culture media and subculture vessels using an autoclave is essential, ensuring that sterilization is not repeated to prevent alterations in composition.

Maintaining a double-door entry system to minimize external contamination, with doors kept closed, sterilizing all culture vessels, including pipettes, using dry heat in a hot air oven, and ensuring proper attire like lab coats, gloves, and masks are worn to minimize contamination risks. Additionally, implementing personal hygiene measures such as tying back hair, avoiding excessive jewellery, keeping nails trimmed, decontaminating used glassware and media through autoclaving before washing and disposal, and regularly wiping laminar airflow platforms and staff hands with alcohol. Finally, autoclave lab coats, gloves, and masks after washing and drying to maintain sterility.

Before use, laminar airflow hoods undergo pre-sterilization using UV light and thorough wiping of surfaces with spirit or alcohol, while periodic fumigation of media preparation and culture maintenance rooms with formalin occurs to control bacterial and fungal contamination, ensuring deactivation of all doors, windows, and electrical circuits during fumigation. Adherence to standardized surface sterilization protocols and immediate cleanup of spills prevent cross-contamination and damage to the work environment, and contaminated

culture flasks are properly disposed of through autoclaving and treatment with chromic acid, followed by washing and drying.

Need for Health and Hygiene at the Workplace

Maintaining health and hygiene standards in a plant tissue culture lab is utmost for several reasons. These labs are dedicated to the propagation and cultivation of plant cells, tissues, and organs under controlled conditions, often involving delicate procedures and sensitive cultures.

- **Preventing Contamination:** Tissue culture work involves handling cells and cultures that can easily become contaminated with bacteria, fungi, or other microorganisms. Maintaining high levels of cleanliness and hygiene reduces the risk of contamination. Contamination in tissue culture lab leads to undesirable results
- **Protecting Personnel:** Some chemicals used in tissue culture laboratories may contain hazardous materials. Proper hygiene practices, including wearing appropriate personal protective equipment (PPE) such as lab coats, gloves, and face masks, help protect laboratory personnel from exposure to these materials, reducing the risk of accidents or adverse health effects. Tissue culture equipment, also needs regular cleaning and maintenance to ensure optimal performance.
- **Promoting Good Laboratory Practices (GLP):** Practicing good hygiene in the laboratory is an essential component of GLP, which encompasses a set of principles to ensure quality and integrity.
- **Preventing Cross-Contamination:** Tissue culture laboratories often handle cultures simultaneously. Without proper hygiene measures, there is a risk of cross-contamination between different cultures, leading to wrong results. Strict adherence to hygiene protocols minimizes this risk and preserves the integrity of individual cultures.

Certain chemicals pose hazards and require careful handling. They can be classified such as flammables, combustibles, explosives, oxidative, toxic materials, compressed gases, corrosive materials, irritants, and sensitizers.

Exposure to UV light can cause acute eye irritation and damage, even without immediate awareness. Always wear appropriate eye protection when using UV lamps.

Proper usage of these chemicals is crucial. Always wear gloves when handling potentially hazardous chemicals and avoid mouth-pipetting them.

ACTIVITIES

Activity-1

Creating a chart of good laboratory practices for plant tissue culture.

Material Required: Large chart paper, Markers, colour sketch pen.

Procedure

1. Explain the importance of following Good Laboratory Practices (GLPs) in a plant tissue culture lab.
2. Divide students into groups and assign each group a section of the chart (e.g., PPE, Chemical Handling, Sterilization process).
3. Each group creates their section, focusing on key practices and guidelines.
4. Review the chart as a class, discussing each section.
5. Hang the chart in the classroom for reference.

Activity-2

Demonstrate the correct way of washing hands using soap water, and alcohol-based hand rubs.

Material Required: Soap, Water, Alcohol-based Hand Rub, Sink, Towels, Poster or Visual Aid

Procedure

1. Explain the importance of hand hygiene.
2. Use the recommended amount of hand rub to prevent skin irritation.
3. Wash hands with water, apply soap, Scrub hands for 20 seconds, covering all surfaces.
4. Use the recommended amount of hand rub to prevent skin irritation.
5. Rinse hands thoroughly under running water.
6. Alternatively, use alcohol-based hand rub, ensuring coverage and allowing it to dry.
7. Dry hands with clean tissue paper or use an air hand dryer.

Activity-3

Demonstrate the use of Personal Protective Equipment while handling chemicals. Prepare a chart, depicting how you can use first aid to handle an emergency at the workplace.

Material Required: Chart Paper, Pictures of PPEs and First Aid

Items, and Writing Material

Procedure

- Follow the safety guidelines desired for the plant tissue culture lab.
- Take a chart paper and paste the pictures of PPEs that one needs to wear while handling chemicals at the workplace.
- Label the PPEs and discuss the usage of each PPE item with the lab manager/teacher.
- On another chart paper, paste the pictures of first aid items that you will use at the workplace.
- Label the first aid items and discuss the importance of each item with your classmates.
- Invite feedback from your classmates and ask if they can suggest some more precautionary measures while handling chemicals at the workplace.

CHECK YOUR PROGRESS

A. Fill in the Blanks

1. Biosafety Level 3 (BL-3) is appropriate for working with agents that have the potential for _____ transmission.
2. Lab benches must be cleaned and decontaminated after completion of work to prevent _____ and maintain a sterile environment.
3. _____ kit must be worn at all times inside the laboratory to minimize risks associated with experimental procedures.
4. Periodic fumigation of media preparation and culture maintenance rooms with _____ helps control bacterial and fungal contamination.

B. Multiple Choice Questions

1. Which biosafety level is appropriate for agents causing moderate risks to human health?
   a) BL-1
   b) BL-2
   c) BL-3
   d) BL-4
2. What should be used to clean workbenches at the end of each day in a laboratory?
   a) Soap
   b) Water
   c) Disinfectant

d) Vinegar

3. Which personal protective equipment is essential to minimize risks associated with experimental procedures?
   a) Lab coats
   b) Gloves
   c) Face masks
   d) All of the above
4. Which chemical poses a fire hazard when in contact with suitable materials?
   a) Xylene
   b) Chloroform
   c) Sodium iodate
   d) Methanol
5. How should acids be diluted in a laboratory setting?
   a) Add water to concentrated acid
   b) Pour acid quickly into water
   c) Add water slowly to the acid
   d) Avoid dilution

C. Subjective Questions

1. Explain Good laboratory practices followed in a plant tissue culture lab.
2. Describe the Need for health and hygiene in workplace.

Glossary

Acclimatization: Gradual adaptation to new environmental conditions, similar to a baby adjusting to new surroundings.

Agar: Gelatinous substance used to solidify the growth medium in tissue culture.

Agarose: Purified form of agar, used as a gelling agent in plant tissue culture media.

Aseptic Conditions: Conditions maintaining extreme cleanliness and sterility to prevent contamination by microorganisms such as bacteria or fungi.

Bioactive Compounds: Chemical substances within plants capable of influencing living organisms, often used in medicine.

BOD Incubator: Equipment ensuring constant temperature and humidity for cultivating biological organisms.

Callus: Mass of undifferentiated cells formed in tissue culture, often used as a starting material for plant regeneration.

Commercialization: Transitioning a product or technology into a business venture or revenue-generating activity.

Culture Medium: Specialized liquid or gel providing nutrients for plant growth in tissue culture.

Disease-Free: Plants devoid of any diseases.

Electronic Balance: Device for accurately measuring small amounts of weight.

Erratic Climate Changes: Unpredictable fluctuations in weather patterns over time.

Ex Vitro: Outside the laboratory setting, such as in a greenhouse.

Explants: Small segments of plant tissue utilized to initiate tissue culture.

Genetic Engineering: Manipulating the genetic material of an organism to improve its characteristics.

Genetic Transformation: Altering the genetic makeup of a plant to enhance certain traits.

Genome Editing: Modifying the DNA of an organism, akin to editing a computer file.

Grouping by Ions: Arrangement of chemicals in stock solutions based on their ions to prevent undesired reactions.

Hardening: Process of acclimating young plants to normal environmental conditions outside the laboratory.

In vitro: Within a laboratory environment, often in a test tube.

Inventory: Comprehensive list of items, such as laboratory supplies.

Laminar Air Flow Cabinet: Controlled workspace where air flows continuously and parallelly to prevent contamination.

Liquid Medium: Culture medium where plant tissues are submerged in liquid for growth instead of on a solid surface.

Macro and Micro Salts: Stock solutions containing essential nutrients crucial for plant tissue culture.

Micropropagation: Producing numerous plants from small fragments of plant tissue.

***Murashige and Skoog* (MS) Medium**: Nutrient blend utilized for plant tissue culture, providing essential minerals and vitamins.

pH Meter: Instrument for measuring the acidity or alkalinity of a solution.

Plant Growth Regulators (PGRs): Chemical substances added to culture media to induce specific growth and development responses in plant tissues.

Polyhouse: Structure constructed from special plastic to shield plants from extreme weather.

Population: Total number of individuals within a specific area.

Precipitation: Formation of solid particles from a solution due to chemical reactions.

Recombinant Technology: Process of combining genes from different sources to create novel genetic compositions.

Refrigerator: Appliance used to maintain low temperatures for storing substances.

Rooting: Process of inducing root formation from plant tissues in tissue culture, necessary for plant development.

Shoot Multiplication: Process of inducing proliferation of shoot structures from explants in tissue culture.

Somatic Embryogenesis: Process of inducing embryo formation from somatic cells in tissue culture, used for clonal propagation of plants.

Space Exploration: Journeying into outer space to study other planets and celestial bodies.

Standard Operating Procedures (SOPs): Established guidelines for performing tasks correctly.

Stock Solution: Concentrated solution containing specific chemicals required for preparing culture media.

Tissue Culture: Growing plants from small plant parts, such as leaves or roots, in a controlled environment with nutrients, without soil.

Answer Key

Unit 1: Introduction to Plant Tissue Culture

Session 1: Plant Tissue Culture Techniques and Its Importance

A. Fill in the Blanks

1. aseptic
2. *in*
3. Totipotency
4. parenchymatous

B. Multiple Choice Questions

1. b
2. c
3. a
4. b

Session 2: Scope and Prospect of Plant Tissue Culture in India

A. Fill in the Blanks

1. $900 million
2. disease free
3. 125
4. Strawberries
5. biofuel

B. Multiple Choice Questions

1. c
2. c
3. c
4. b
5. a

Unit 2: Laboratory Setup and Equipment for Plant Tissue Culture

Session 1: Laboratory Setup and Its Component

A. Fill in the Blanks

1. microbial presence
2. Primary Hardening facility
3. Growth Room
4. Inoculation Room
5. Media Preparation

B. Multiple Choice Questions

1. b
2. a
3. d
4. a
5. c
6. c

Session 2: Laboratory Equipment and Sterilization Process

A. Fill in the Blanks

1. Autoclave
2. 121°C
3. acidity or basicity
4. 0.2
5. microorganisms

B. Multiple Choice Questions

1. d
2. c
3. d
4. d
5. b

Unit 3: Preparation and Storage of Culture Media for Plant Tissue Culture

Session 1: Types of Tissue Culture Nutrient Medium

A. Fill in the Blanks

1. MS medium
2. Woody Plant Medium
3. Na_2EDTA
4. Meta-topolin (mT)

B. Multiple Choice Questions

1. b
2. d
3. d
4. b
5. a

Session 2: General Methodology of Media Preparation

A. Fill in the Blanks

1. Regent
2. Sachets
3. Cytokinins
4. Micromolar
5. 1000 ml

B. Multiple Choice Questions

1. b
2. c
3. a
4. c
5. b

Session 3: Preparation of Buffers and Solutions

A. Fill in the Blanks

1. Silver Chloride
2. pH 4, pH 7, and pH 10
3. 5.5 to 5.8
4. 0.3-0.5
5. buffered

B. Multiple Choice Questions

1. b
2. a
3. c
4. a
5. b

Unit 4: Maintenance of Aseptic Condition in Laboratory

Session 1: Process of Sterilization and Storing the Culture Medium

A. Fill in the Blanks

1. 160
2. 15
3. 2600
4. 0.5-1.0
5. 70

B. Multiple Choice Questions

1. a
2. c
3. c
4. b
5. c

Session 2: Biosafety Practices and Cleanliness in a Plant Tissue Culture Laboratory

A. Fill in the Blanks

1. aerosol
2. cross-contamination
3. Personal Protective Equipment (PPE)
4. formalin

B. Multiple Choice Questions

1. b
2. c
3. d
4. c
5. c

List of Credits

DAAH, PSSCIVE, Bhopal

- Figure 1.9
- Figure 1.3
- Figure 1.4
- Figure 10
- Figure 11

Dr. (Mrs.) Vinod Kataria, JNVU, Jodhpur, Rajasthan

- Figure. 1.2
- Figure 1.5

Dr. Maneesh Mishra, ICAR- CISH, Lucknow, Uttar Pradesh

- Figure 2.1
- Figure 2.2
- Figure 2.3
- Figure 2.4
- Figure 2.5
- Figure 2.6
- Figure 12
- Figure 2.8
- Figure 13
- Figure 2.10
- Figure 2.11
- Figure 2.12
- Figure 14
- Figure 2.14
- Figure 15
- Figure 2.16