PLANT TISSUE CULTURE: GENERAL TECHNIQUES

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***Abstract*: In order to regenerate and propagate complete plants, the technique of plant tissue culture is used to grow isolated plant cells, tissues, and organs in axenic conditions (in vitro). All plant cultures, including callus, cell, protoplast, anther, meristem, embryo, and organ cultures, are frequently referred to collectively as "tissue cultures" in this context. In order to answer crucial fundamental questions in plant biology, plant cell, tissue, and organ culture techniques were created and established as an experimental necessity. However, they now serve as very valuable biotechnological tools for a number of crucial applications, including the commercial micropropagation of various plant species, the production of disease-free plant materials, the production of haploid and double haploid plants, and the induction of epigenetic or genetic variation. Besides the main sources of carbohydrates, vitamins, and plant growth regulators, a medium is a mixture of inorganic salts and organic substances used to nourish plant cultures.**

***Keywords*: Micropropagation, Plant tissue culture, Tissue & Organ culture, In vitro plant culture, Material & methods, Application.**

1. PLANT TISSUE CULTURE

1.1 INTRODUCTION

Plant biotechnology is not complete without plant tissue culture. In addition to allowing for the mass reproduction of elites, it also gives people the ability to reproduce and regenerate brand-new plants using genetically altered cells. The promising plant created in this way can be easily cloned in cultures under aseptic circumstances. Tissue Culture is widely used in –

- Getting plants free of disease;
- Propagating plants that are hard to replicate quickly.
- Hybridization of somatic cells.
- The genetic enhancement of industrial plants.
- Obtaining haploid plants that are androgenic and gynogenic for breeding programs.

Tissue culture is increasingly being used as a substitute for vegetative plant propagation. Bacterial and fungal infections are typically absent in plants that are grown in vitro. In cultures, virus eradication and plant care in the virus-free stage can be accomplished quickly. Three basic techniques are typically employed in tissue culture:

- Micropropagation through axillary bud multiplication
- Organogenesis.
- Somatic embryogenesis[1].

The most effective and widely utilized strategy at the moment is improved shoot multiplication from axillary bud. The axis of leaves contains axillary buds. The dormancy of the axillary buds in tissue culture can be disrupted by employing the right concentration of cytokinin or a combination of cytokinin and Auxin. They become shoots after the dormancy is disturbed. They can be induced to proliferate very quickly by utilizing substrates that include plant growth regulators in the right proportions. Today's plant tissue cultures are carried out in aseptic settings with filtered air. Starting materials (explants) must be surface sterilized in chemical solutions (often alcohol) due to the inherent contamination of living plant materials from the environment with microbes on their surfaces (and occasionally interiors). Today, mercuric chloride is utilized to sterilize plants despite being hazardous to use and challenging to discard. Explants are then typically positioned on top of a solid culture medium, though this practice is occasionally used, especially when cell suspension cultures are desired. Inorganic salts, together with a few organic minerals, vitamins, and plant hormones, make up the majority of the ingredients in solid and liquid media. Liquid media are converted into solid media by adding a gelling agent, often pure agar[2]. The shape of the tissues that develop from the initial explant is significantly influenced by the medium's composition, particularly the plant hormones and the nitrogen supply (nitrate as opposed to ammonium salts or amino acids). For instance, an excess of auxin frequently causes the growth of roots, but an excess of cytokinin might produce shoots. The form of the callus will depend on the plant species and the makeup of the medium, although a balance of auxin and cytokinin will frequently result in an unstructured proliferation of cells. To allow for growth or to change the morphology of the culture, parts are frequently cut off and transferred to new media (subcultures). The tissue cultivator's expertise and experience are crucial in determining which parts to keep and which to discard. When shoots begin to appear in a culture, they can be cut off and rooted with auxin to create plantlets, which can then be planted in potting soil and grown in a greenhouse as regular plants[3].

1.2 HISTORY OF PLANT TISSUE CULTURES

➢ Aseptic culture of cells, tissues, organs, and their constituent parts is known as plant tissue culture. In vitro, under specified chemical and physical conditions.

➢ the underlying principles of plant tissue culture was put out by Gottlieb Haberlandt in his 1902 address to the German Academy of Science regarding his studies on the culture of single cells.

- In the past, Henri-Louis Duhamel du Monceau (1756) was the first to conduct research on plant wound healing through the creation of spontaneous calluses (an unorganized mass of cells) on decorated elm plants.
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- Vochting (1878) proposed that Polarity be a crucial characteristic that directs the development of plant pieces.
- He saw that a segment of a stem's top portion always produced buds, while the base region always produced callus or roots.
- German botanist Gottlieb Haberlandt created the idea of Tradescantia isolated cells being cultured under artificial conditions in 1902. Although his attempt to get the cells to divide failed.

- He failed because even Auxin had not been found by that point. But he gave plant physiology a Foundation.
- He discussed how to grow mesophyll cells from Lamium purpureum, Eichhornia Crassipes, Ornithogalum, and pumonaria, as well as epidermal and hair cells.

- Cell maintained life for 3–4 weeks. Haberlandt is known as the Father of Tissue Culture as a result of this Endeavour. Most significantly, he proposed the idea of totipotency.
- Attempts at organ culturing were made from 1902 to 1930. Hannig (1904) successfully cultivated certain crucifer embryos in mineral salt and sugar solutions.

- On the surface of a medium containing IAA, which promoted cell division, Simon (1908) effectively recreated a bulky Callus, buds, and roots from a poplar tree.
- The identification of auxin as a natural growth regulator and the understanding of the significance of B-vitamins in plant growth were the two significant discoveries discovered in the middle of the 1930s that greatly influenced the development of the plant tissue culture technology.
- In 1934, Gautheret grew cambium cells from a few different tree species (Salix capraea, Populus nigra), recording that they proliferated for a few months in Knop's solution containing glucose and cysteine hydrochloride.
- The first true plant tissue cultures were Obtained by Gautheret from cambial tissue of Acer pseudoplatanus.
- Using an agar-solidified medium of Knop's solution, glucose, and cysteine hydrochloride, he also had success with identical explants of Ulmus campestre, Robinia pseudoacacia, and Salix capraea.
- Gautheret developed the first continuously expanding tissue cultures from carrot root cambium in 1939.
- White (1939a) reported the establishment of Similar cultures from tumor tissue of the hybrid Nicotiana glauca x N. langsdorffii.

- Next, P.R. White (1939) and R.J. Gautheret simultaneously announced the possibility of cultivating plant tissues for an endless amount of time (1939).
- The modern media composition was established by Gautheret and White between 1930 and 1940. •Subsequently, in-depth work by Raghavan and Torrey (1963), Norstog (1965), and others resulted in the invention of synthetic media for the culture of younger embryos.
- From 1940 to 1970, appropriate nutritional media for the cultivation of plant cells, tissue, protoplasts, anthers, roots, and embryos were produced. I
- plant in vitro morphogenesis, which is the regeneration of an entire plant from cultured tissue. Was always completed successfully.
- Skoog and Miller proposed the idea of hormonal regulation of organ creation in 1957.
- Morishige played a key role in establishing in vitro culture techniques as a practical and feasible method for horticultural species propagation. He contributed significantly to the technique's popularization by creating standardized techniques for the in vitro cultivation of numerous species, including ferns, leaf, flower, and fruit plants.
- In 1959, F. Skoog, C.O. Miller, and colleagues made the discovery of kinetin, which paved the door for plant tissue culture. They also demonstrated how to induce the regeneration of shoots in tobacco calluses.
- E. Cooking created a method to isolate protoplasts in large quantities using a fungus enzyme derived from Myrothecium sp. In the 1960s.
- Jones et al. developed a microculture technique in 1960 for cultivating individual cells in suspended Drops in conditioned media.
- Braun created the first plant from a mature plant cell in 1959[4].

INDIAN HISTORY

- In India, Panchanan Maheshwari, who is known as the country's "Father of Embryology," began working on tissue culture in the middle of the 1950s at the Department of Botany (University of Delhi).
- Under the direction of P. Maheshwari, the University of Delhi's Botany School became heavily involved in the in vitro production of flowering plants' reproductive parts in the 1960s.
- Kanta invented test-tube fertilization and intraovarian pollination in 1960.
- For morphogenic investigations including ovary, embryo, endosperm, ovules, etc., several tissue culture approaches were used.
- Through anther and pollen cultures, Sipra Guha Mukherjee and S.C. Maheshwari (1966–1977) created the haploid for the first time at the University of Delhi.
- Maheshwari and Guha created the first haploid plants from pollen grains in 1964 by cultivating several Datura species.

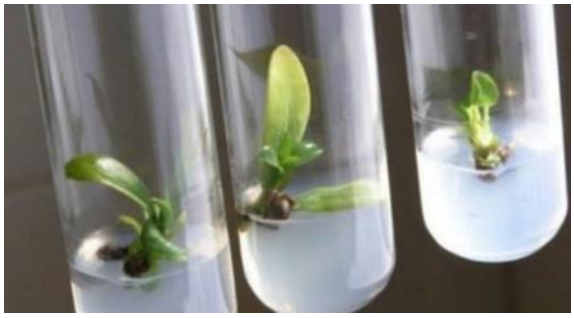
1.3 IMPORTANCE OF PLANT TISSUE CULTURES

- Starting with a single explant, many plantlets can be produced in a small amount of time and space.
- Because taking an explant typically does not kill the mother plant, it is safe to clone rare and endangered plants.
- Choosing desirable features straight from the in vitro growing setup is simple, which reduces the amount of area needed for field testing.
- A plant tissue culture line can provide a year-round supply of young plants once it is established.
- The time needed is significantly reduced; the entire seed development life cycle is not necessary. Rapid propagation is possible for species that have long generation times, little seed production, or easily non-germinating seeds.
- Plants grown in vitro are often devoid of bacterial and fungal infections. Removing viruses from plants and keeping them virus-free. This makes it easier for plants to travel across international borders.
- It is possible to freeze plant tissue banks and subsequently regenerate them using tissue culture. It keeps the pollen and cell collections that can be used to grow plants[1].

1.4 TYPES OF PLANT TISSUE CULTURES

❖ **Embryo culture**

The process of growing embryos from seeds and ovules in a nutritional media is known as embryo cultivation. In embryo cultivation, the plant either grows directly from the embryo or indirectly by developing a callus, which is followed by the development of branches and roots.



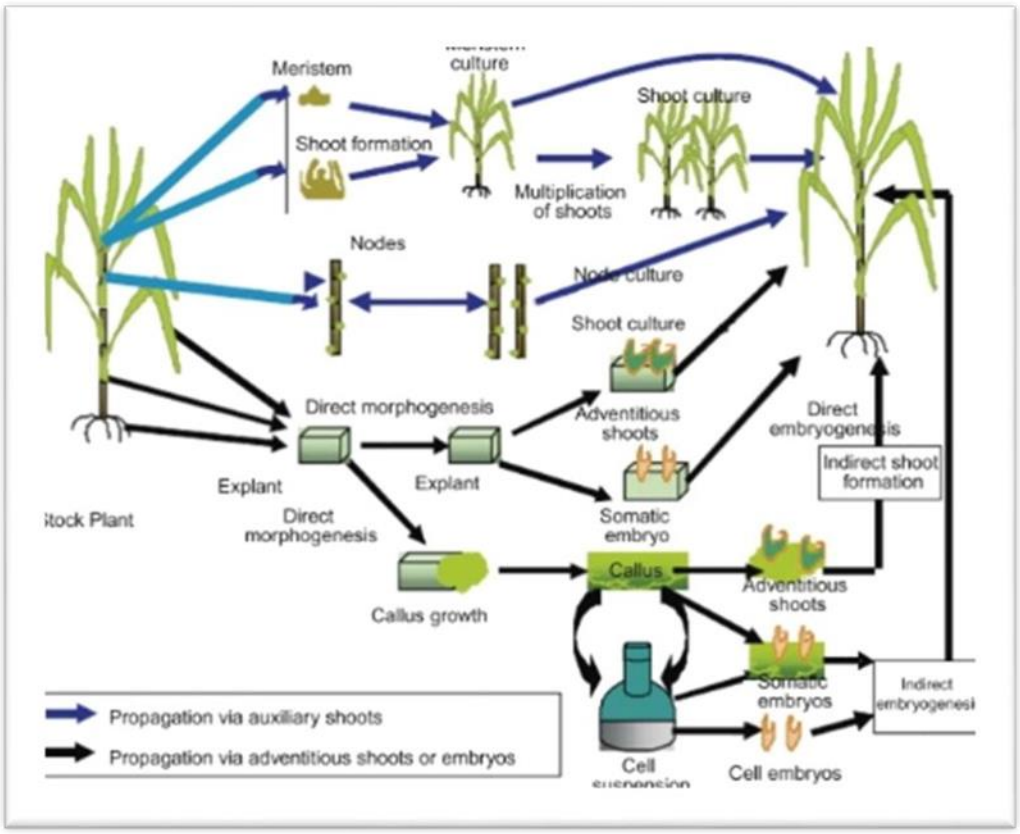
The method was created to induce germination of uncommon species and haploid plants, assess the viability of seeds, and break seed dormancy. The plant has significant economic importance both as a source of lumber and for use in medicine. By providing a means of propagating exceptional individuals in situations where the selection and improvement of the natural population are challenging6, this technology has a significant application in forestry[6].

❖ **Callus culture**

Limiting plant material may be amplified more easily using callus cultures. Additionally, plant regeneration from Calli allows for the isolation of uncommon soma clonal variations that are either the product of genetic diversity already present in somatic cells or are caused by the application of environmental stimuli, such as growth factors added to the cultured cells, in vitro[7].

❖ **Organ Culture**

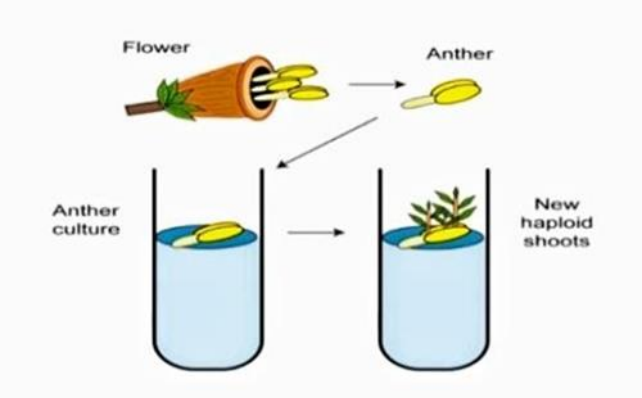
The term “organ culture” refers to the cultivation of organs or plant parts in artificial conditions or in isolated media. Any plant component, including the shoot (for shoot tip culture), root (for root tip culture), leaf (for leaf culture), and flower, may be used as an explant in organ culture (for another, ovary, ovule cultures). In investigations of the dependency on growth regulators and other growth factors, organ culture has proven to be quite dependable. Additionally, it aids in enlarging the potential scope of agricultural and horticultural advancements.



Meristematic culture, shoot tip, nodal culture of distinct lateral bud, isolated root, and embryo culture are the most significant organ culture types for in vitro plant propagation. Numerous plants, such as Fritillaria unibracteata, have created in vitro organ culture and metabolic composition of chemicals. By using the organ culture technique, the plant species can be quickly propagated from small cuttings of the bulb, and cultured bulbs can be harvested after some time in MS medium, where growth rate, alkaloid, and microelements have been reported to be many times (3050) more abundant in cultured bulbs. Therefore, it has been demonstrated that organ culture is a very important strategy for enriching alkaloid in plants[8].

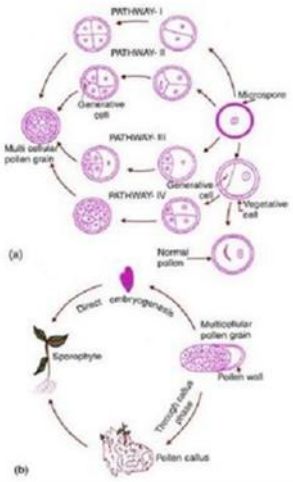
❖ **Anther culture**

In order to produce haploid and diploid plants, another culture refers to the regeneration of plants from haploid microspore cells[9]. A method of cultivating plants that causes immature pollen to divide and develop into tissue (either callus or embryonic tissue) on solid or liquid substrates. When pollen-containing anthers from a flower are removed and placed in a culture medium, some microspheres survive and form tissue. If callus tissue develops, it is placed in a hormone solution that will encourage it to differentiate and develop shoot and root tissue. If embryonic tissue develops, it is placed in a medium that is favorable for shoot and root development.



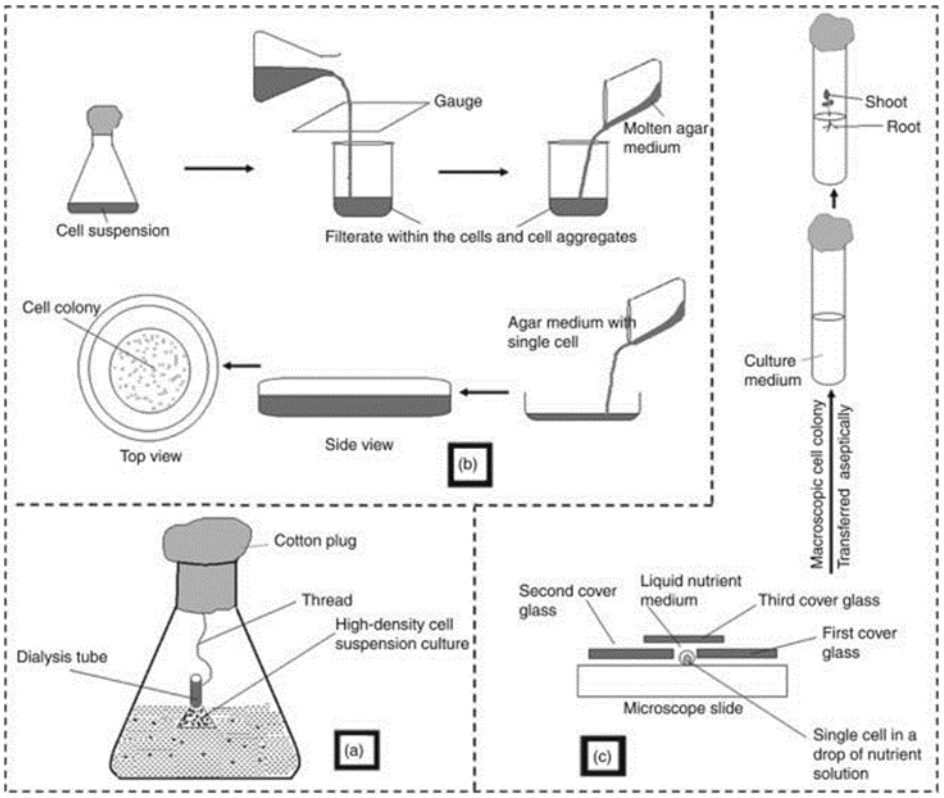
❖ **Pollen culture**

Pollen culture is an in vitro method that involves squeezing the pollen grains (ideally at the microscope stage) from the intact anther and cultivating them on nutritional media without creating male gametes.



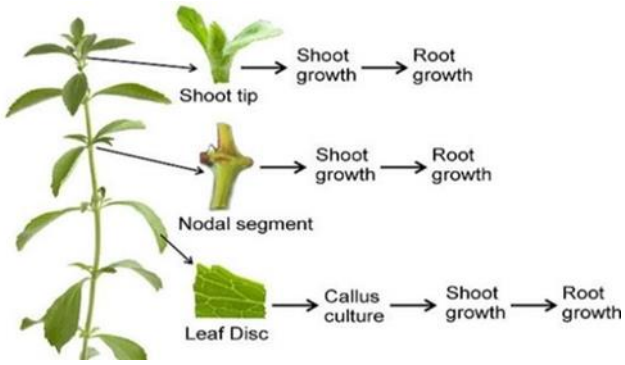
❖ **Suspension culture**

Cultures of Calli, which are collections of undifferentiated cells that proliferate on the surface of solid culture medium, and cell suspension cultures, in which cells grow in liquid culture media while being constantly shaken, are examples of the more prevalent plant cell culture methods. Subcultures of cell cultures must be repeated at intervals that depend on the pace of cell growth. Callus cultures, which develop more slowly than cell suspension cultures, enable the long-term preservation of plant cell lines and give the experimenter a supply of fresh plant material in the event that the cell suspension cultures get contaminated with microorganisms. Plant cell suspensions may also be cryopreserved as an alternative[10].



❖ **Explant culture**

There are many different types of seed plants, including trees, herbs, and grasses, which display the fundamental morphological components of a root, stem, and leaves. The most adaptable sort of tissue is parenchyma. They have the capacity to multiply and expand.



1.5 PLANT IN VITRO CULTURE TECHNIQUE

Plant in vitro technologies provide promise in three key areas: somatic cell genetics, micropropagation, and the creation of transgenic plants.

Micropropagation -
To create superior clonal plants, tissue culture propagation, also known as micropropagation, is utilized (Smith 1990). The ability to quickly reproduce novel genotypes on a wide scale and the utilization of little original material are considered to be the key benefits.

Somatic cell genetics-
The main contribution of in vitro techniques, namely somatic cell genetics, to plant breeding is haploid generation and somatic hybridization.

Transgenic plants-
For many plant species, the expression of mammalian genes or other plant genes has become commonplace. One of the effective methods has been modified to be resistant to herbicide as well as to insects, viruses, and other infections.

1.6 ADVANTAGES OF PLANT TISSUE CULTURE

- Starting from a single explant, several plantlets may be generated in a short amount of time and space.
- When a tissue is isolated in vitro, the nature of some of these correlative controls can be ascertained. In a living plant, the behavior of each tissue is strongly influenced by correlative controls imposed by the rest of the plant.
- The creation of precise replicas of plants with desired qualities, such as those that yield excellent fruits or flowers.
- To grow mature plants soon.
- The growth of many plants without seeds or the pollinators required to create seeds.
- The regrowth of whole plants from genetically altered plant cells.
- Growing plants in sterile containers, which considerably reduces the risk of diseases, pests, and pathogens spreading when they are transferred.
- The growth of plants from seeds that would normally have very little chance of germinating and developing, such as nepenthes and orchids[1].

2 MICRPROPAGATION

2.1 PRINCIPAL OF MICROPROPAGATION

Plant micro propagation is an integrated process in which isolated, sterilized cells, tissues, or organs of chosen plants are incubated to create numerous clone plantlets in an environment that promotes growth. The technique of cloning isolated demonstrated the fact that somatic cells, under appropriated conditions, can differentiate to a whole plant. This potential of a cell to grow and develop a multicellular organism is termed cellular totipotency. A This potential of cells or tissues to form all cell types and regenerate plant Micropropagation[11].

2.2 GENERAL TECHNIQUE OF MICROPROPAGATION

The various steps or stages involved in the complex process of in vitro clonal propagation or micropropagation are summarized in four stages. The four stages of overall micropropagation were recognized in 1978 by Morishige. Stages I to III were considered under in vitro conditions and stage IV under greenhouse conditions. Stage 0 was introduced in the micro-propagation system by Debergh and Mane in 1981.

❖ **Stage 0: Stock/Elite Plants Selection**

The initial stage in micropropagation is choosing stock or elite plants that are suitable for mass multiplication. Following selection, the stock/elite plants are kept for three months to begin the culture process in a controlled environment with watering, low humidity, and no systemic microbial infections.

❖ **Stage I: Aseptic Culture Establishment**

Explants chosen in Stage 0 are developed on a clearly defined culture medium in this stage and are then ready for inoculation after being treated with an appropriate sterilizing agent. Depending on the degree of surface contamination, the axillary bud, shoot tip, and meristem are surface sterilized using different chemicals such 5% sodium hypochlorite, 0.1% mercuric chloride, or 70% alcohol with varying contact periods. The explants are inoculated onto the MS medium (or any other suitable basal medium) following surface sterilization. The MS medium is supplemented with different growth regulators, vitamins, and sucrose. A culture medium with cytokinin's (1-3 mg/L 6-benzyl amino purine) and less auxin (indole-3-acetic acid) was utilized for micropropagation. Auxin 2,4-dichlorophenoxyacetic acid is beneficial because it inhibits organogenesis and promotes the development of calluses. The cultures are cultured for 16 hours at a light intensity of 3000-10,000 flux.

❖ **Stage II: Multiplication of the Explant**

Through the regeneration of explant-derived shoots, explants are multiplied over an extended period of time. Sometimes the apical shoots only produce a single shoot, and this single shoot is then utilized to excise a number of nodal explants. Nodal explants are further inoculated for multiple shoot proliferation using cytokinin-rich medium. By using organogenesis directly on explants or somatic embryogenesis, several shoots may also be created throughout this procedure. In a matter of 4 to 5 weeks, each explant generates 5 to 6 shoots. Single explants would generate 510–612 plants in a year if all the plants survived.

❖ **Stage III: Germination of Somatic Embryo or Rooting of Regenerated Shoots**

Multiple shoots formed during stage II cause rooting in new media. Individually separated shoots are inoculated (with auxins) into the rooting media, and in certain instances rooting is directly stimulated in the soil under circumstances of high wetness. The rooted plants are extremely delicate and sensitive to dampness. When it comes to somatic embryos, germination is permitted to create the plantlets before they are planted in the ground. The plantlets are gradually introduced to the soil after going through the hardening procedure. Before being transferred to the soil, the hardening medium should be either pearlite, peat, or vermiculite, which can contain a significant amount of moisture and be kept at high humidity levels.

❖ **Stage IV: Hardening**

The plantlets of stage III are hardened to external soil conditions from their in vitro environments at this stage. This entails the plants changing from their heterotrophic state in in vitro culture settings to an autotrophic state as a result of their increased resistance to stress, wetness, and disease. Plantlets need to be shielded from direct sunshine, and the relative humidity should be reduced gradually. The plantlets establish strong roots during this time of acclimation, and the aerial tissues also produce cuticular wax. After that, the plantlets are ready to be moved to open areas. When vitrification occurs, it may sometimes be seen in in vitro experiments where particular species or shoots seem brittle, shiny, and wet. Stomatal dysfunction, inadequate vascular bundle development, and irregular wax quality all contribute to vitrification, which causes plantlets to die. Growth inhibitors, bottom chilling of culture tubes, high concentrations of agar (1%), and hydrolysate chemicals can all be used to treat this condition[12].

2.3 ADVANTAGES OF MICROPROPAGATION

Micropropagation has a number of advantages over traditional plant propagation techniques: The main advantage of micropropagation is the production of many plants that are clones of each other.

- Plants that are free of disease can be produced via micropropagation.
- When cuttings or seeds take a long time to germinate or grow, micropropagation creates rooted plantlets that are ready for growth, saving the grower's time.
- It can create thousands of propagules at an incredibly high frequency rate, whilst conventional approaches might only manage a small portion of this amount.
- It is the only approach that can successfully regenerate genetically altered cells or cells following protoplast fusion.
- It may be used to increase the number of plants that generate seeds in uneconomical quantities, sterile plants that cannot create viable seeds, and plants that cannot store their seeds.
- Compared to plants generated using more traditional techniques, such as seeds or cuttings, micropropagation frequently results in stronger plants, which accelerates development.
- The majority of orchids and other plants with extremely tiny seeds may be reliably cultivated from seed in sterile culture.
- The propagules may be held longer and in a smaller space, and more plants can be grown per square meter.

2.4 DISADVANTAGES OF MICROPROPAGATION

Micropropagation is not always the perfect means of multiplying plants, conditions that limits its use include:

- It can cost more than 70% of the total cost in labor, making it exceedingly costly.
- After micropropagation, a monoculture is created, which results in a lack of overall disease resistance since all offspring plants could be susceptible to the same illnesses.
- A plant sample that has been infected can generate contaminated offspring. If the stock plants are thoroughly examined and screened to avoid cultivating plants that are contaminated with a virus or fungus, this is unusual.
- Not all plants can be successfully tissue cultured, often because the proper medium for growth is not known or the plants produce secondary metabolic chemicals that stunt or kill the explant[1].

3 MATERIAL AND METHODS

3.1 LAB REQUIREMENTS

The current study was conducted at the Arid Forest Research Institute in Jodhpur (Rajasthan). The experimental materials used in the current study on Dendrocalamus stritus micropropagation.

APPARATUS-

1. pH Meter
2. Microwave oven
3. Electronic Weighing Balance
4. Refrigerator
5. De-freeze
6. Oven
7. Autoclave
8. Laminar Air Flow
9. Incubator

CHEMICALS-

1. Ammonium Nitrate
2. Potassium Nitrate
3. Boric Acid
4. Potassium Di Hydrogen ortho Phosphate
5. Potassium Iodide
6. Sodium Molybdate di hydrate
7. Cobaltous Chloride
8. Calcium Chlorine
9. Magnesium Sulphate
10. Manganese Sulphate
11. Zink Sulphate
12. Cupric Sulphate
13. Sodium Ethylene di amine tetra acetic acid
14. Ferrous Sulphate
15. Thiamine HCl
16. Nicotinic Acid
17. Pyridoxine
18. Glycine
19. Myoinositol
20. Sodium Hydroxide
21. Hydro Chloric Acid
22. Sucrose
23. Agar-Agar
24. Citric Acid
25. Glutamic Acid
26. Adenine Sulphate Di hydrate
27. Asparagine
28. Arginine
29. Mercuric Chloride
30. Ascorbic Acid
31. Sodium Hypochlorite
32. Benzyl Amino Purine (BAP)

33. Indole Acetic Acid (IAA)
34. A-Naphthalene Acetic Acid (NAA)
35. 2,4-Di chlorophenoxyacetic acid(2,4-D)

3.2 PLANT TISSUE CULTURE MEDIA COMPONENTS

The creation of plant tissue culture medium can have a significant impact on the growth and development of plants. For a wide range of plant species, many various formulations have been created over the years, although for the majority of plant tissue culture operations, a few are more frequently utilized. Although the Morishige & Skoog formulation (1962) is by far the most widely utilized base medium today, other efficient media are routinely employed as well. These include those mentioned in "Gamborg's B-5" by Gamborg et al. (1968), "Woody Plant Medium" by Lloyd and McCown (1980), "Schenk and Hildebrandt," "Nitsch and Nitsch," and White's (1963) reports. These media formulations can be created from salt and vitamin stock solutions in liquid form or bought as powder packets that have already been combined.

Water, nutritional salts (micro and macronutrients), vitamins, amino acids, carbohydrates, gelling agents, growth regulators (hormones), and other organic supplements are among the various ingredients and additives that may be employed in plant micropropagation media. The breakdown of each component category is as follows:

Water-
Water makes up the majority (90+%) of the components in plant culture medium. To ensure that no minerals or other pollutants have an impact on plant growth, it is crucial to use ultrapure water. Reverse osmosis or distillation are both used to achieve this.

Nutrient salts-
The "macro" and "micro" components required for plant development are provided by nutrient salts. Macronutrients, such as nitrogen, phosphorus, potassium, calcium, magnesium, and Sulphur, are needed in higher concentrations (1–60 mM). Micronutrients, such as iron, manganese, zinc, boron, copper, molybdenum, cobalt, and iodine, are needed in lesser amounts (0.1–100 M). While added to some medium, sodium and chlorine are not thought to be necessary for plant development. In order to make macro- and micronutrients more accessible to plants, salts are often added to the medium. Calcium nitrate, potassium chloride, and magnesium sulphate are some examples of nutritional salts. In order to increase availability and prevent precipitation of such elements, iron and zinc are occasionally supplied in chelated form. The chelating agents EDTA or EDDHA are frequently used.

Vitamins-
Unlike plants grown on soil or other substrates, plants produced in vitro frequently lack the capacity to synthesis vitamins. To enhance plant tissue culture medium with substances that work to boost certain metabolic processes, a variety of vitamins are added. Thiamine, nicotinic acid, pyridoxine, and myo-inositol are a few of the vitamin supplements that are typically utilized at rates of 0.1–10 mg/L.

Amino acids-
Because they are a readily available supply of nitrogen that plants may sometimes more easily absorb than from inorganic sources, some amino acids are added to plant tissue culture medium and can be useful for plant cell development. The medium may include different amounts of single amino acids such glycine, glutamine, or adenine as well as amino acid combinations like casein hydrolysate.

Carbohydrates-
Unlike in the natural environment, most in vitro plants are unable to synthesize their own carbohydrates from light, water, and carbon dioxide. The medium must provide a source of carbohydrates for plant development. Sucrose is the most often used carbohydrate source, but 20–30 g/L amounts of glucose, fructose, and maltose are also utilized. The impact of the kind and quantity of carbohydrates utilized in the medium varies depending on the species of plant.

Gelling agents-
A gelling agent, or a combination of agents, is often added to plant tissue culture medium. Gelling agents are used to give the plant that is being cultivated physical support. Agar, which is generated from the red alga Gracilaria, is a common gelling agent, while gellan gum, which comes from the bacteria Sphingomonas, is often employed on its own or in conjunction with agar. In particular for pollen production, agarose (extracted from some forms of red seaweed) is employed less frequently. Testing is advised since different plant species could grow better when employing a particular gelling chemical or a combination of many. Sometimes the brand or the quality of the gelling chemical might affect how plants react. If no gelling agent is utilized, as is the case for suspension cell cultures, either constant shaking of the culture on a shaker table or, in the case of shoot cultures, the use of some sort of support system (such as a paper "raft") may be required.

Growth regulators-
To obtain the required growth properties for the target plant species/cultivar, plant growth regulators (PGRs) are frequently added to plant tissue culture medium. PGRs fall into one of four major categories: auxins, cytokinin's, gibberellins, or abscisic acid. The most prevalent are auxins and cytokinin's, which are frequently employed in conjunction with one another. While cytokinin's

encourage axillary and adventitious shoot production and growth, auxins tend to promote root and callus initiation and growth. Abscisic acid can boost and improve the general quality of somatic embryogenesis, and gibberellic acid is utilized to encourage shoot elongation.

The most frequently used auxins Indoleacetic acid (IAA), indole butyric acid (IBA), naphthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4- D) are employed in plant tissue culture medium at rates of 0.01 to 10 mg/L. Benzyl adenine (BA), kinetin, and zeatin are three commonly utilized cytokinin's that are employed in concentrations between 0.1 and 10 mg/L. The majority of PGRs may be autoclaved alongside other media components, although it is advised to filter-sterilize and add PGRs after autoclaving if a critical concentration is needed.

Since PGRs are utilized in culture medium in microscale quantities, stock solutions (often with a predetermined concentration of X mg/mL) are used to make it easier and more precise to measure out the required amount. Prior to dilution for the final concentration of the stock solution, the majority of PGRs should be dissolved into a suitable solvent (such as ethanol, DMSO, sodium hydroxide, hydrochloric acid, etc.). Stock solutions should be frozen or kept in a refrigerator.

Other organic supplements and antioxidants-
There are many other organic substances that can support plant development in tissue cultures. These include activated charcoal, malt extract, protein hydrolysates, and coconut milk (AC). When plant tissues create oxidized phenolic compounds or other contaminants that may over time turn harmful, activated charcoal is occasionally added to plant cultures. Toxic substances that might ordinarily hinder development can be absorbed by adding AC to the medium at a rate of 0.5–5 g/L.

Reactive oxygen species (ROS), which can lead to oxidative damage to plant tissues, can be prevented or reduced by supplementing plant tissue culture medium with antioxidants and antioxidant-like substances. There is evidence that several substances, including ascorbic acid, citric acid, glutathione (in its reduced form), lipoic acid, glycine betaine, D-tocopherol (vitamin E), salicylic acid, and polyvinylpyrrolidone (PVP), have the potential to reduce ROS formation. However, their effects differ significantly between different plant species. In the pretreatment and preculture media, the National Laboratory for Genetic Resources Preservation in Fort Collins, Colorado, treats Vitis single-node micro cuttings and shoot tips with a combination of 1 mM ascorbic acid, 1 mM glutathione (reduced form), and 0.1 mM salicylic acid, respectively (Bettoni et al. 2019). Due to its antioxidant benefits, this therapy has been demonstrated to greatly increase shoot regeneration following cryopreservation. Similar findings were made by Uchendu et al. (2010) who discovered that increasing the regeneration of cryopreserved shoot tips by adding reduced glutathione, lipoic acid, or glycine betaine to various stages of the cryopreservation procedures.

3.3 PREPARATION OF THE STOCK SOLUTIONS

It is challenging to measure and combine all the ingredients right before making the medium. It is a laborious and time-consuming task. Again, it is highly challenging to weigh some ingredients that are utilized in very small quantities for 1 liter of medium if 100 ml of medium is to be created. Therefore, it is convenient to prepare concentrated stock solutions of macro and micro salts, vitamins, amino acids, hormones, and other substances. All stock solutions should be stored in a refrigerator and visually inspected for microorganism contamination or ingredient precipitation.

Sterilization

It is very important to maintain aseptic environment during the in vitro culture of plant cells and tissues. Following is some of the methods adopted for sterilization:

(a) **Sterilization of Glassware-** The glassware can be sterilized in a hot air oven at 160-1800C for 2-4 hours.

(b) **Sterilization of instruments-** The metallic instruments are incinerated by dipping them in 75% ethanol followed by flaming and cooling.

(c) **Sterilization of nutrient media-** The culture medium is put into glass containers, sealed with plastic lids or cotton plugs, and autoclaved at 15 psi for 30 minutes to sterilize them. The vitamins, plant extracts, amino acids, and hormones are denatured by autoclaving; as a result, the solutions of these substances are sterilized using Millipore filter paper with 0.2-millimeter diameter pores. Juvenile material may often be surface sterilized without much difficulty. However, if older trees are employed, contamination of explants can occasionally be a severe issue, unless the tree produces juvenile sprouts, since it is the first and fundamental material for tree breeders when selection is done.

Rare circumstances, insects will drop spores on trees that have been cultivated in fields. Insecticides and fungicides can be sprayed on these trees to reduce contamination. Explants can then be protected from insects by being placed in bags made of transparent film before being collected. A variety of microbial pollutants are present on the surface of plant parts. Before placing the tissue on the nutritional medium, it must be extensively surface sterilized to eliminate this source of infection. Various sterilizing chemicals have been employed to clean plant tissues. In most instances, hypochlorite treatments have shown to be overly effective. Plant

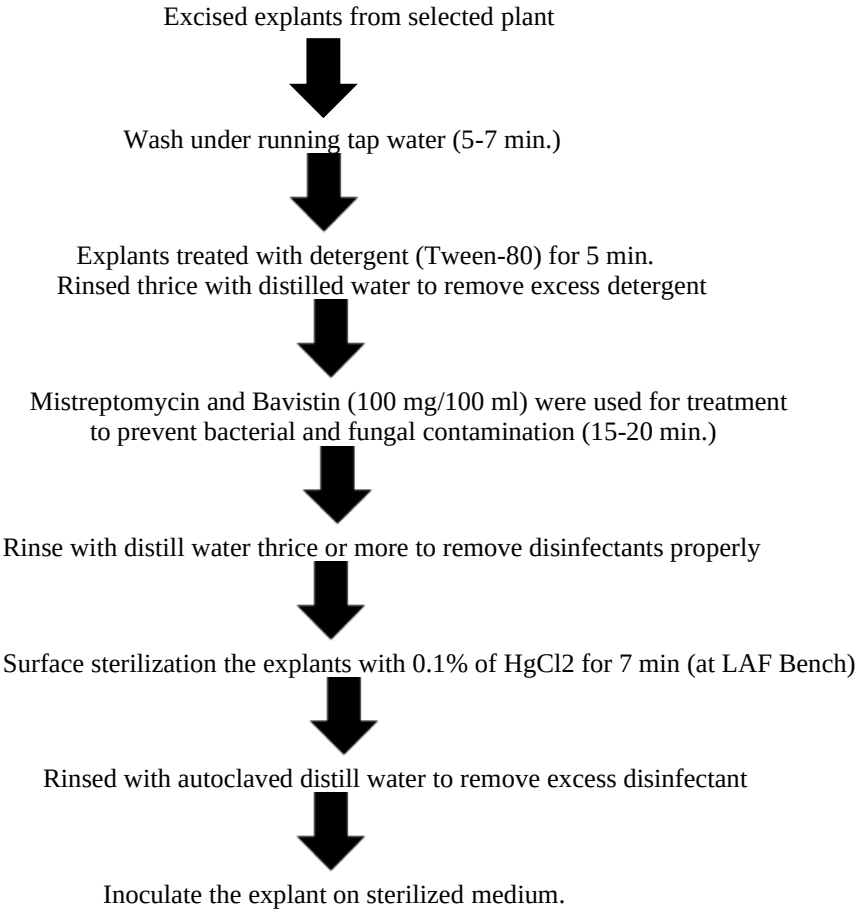
tissues have also been surfaced sterilized using ethyl and isopropyl alcohol. Explants were cleaned in distilled water to get rid of any dust before being cleaned in detergent solution and surface sterilized for five minutes in a 0.1% solution of HgCl2, NaCl. Nodal segments were once again rinsed with sterile distilled water to eliminate the sterilant.

(d) Sterilization method of Explants-

Table: - Some of the agents used for surface sterilization of explants

Disinfectant	**Disinfectant**	**Duration of treatment**
Ethyl alcohol	75-96%	1-10min
Mercuric chloride	0.1-1.0%	2-10min
Sodium Hypochlorite	0.5-5%	5-30min

A flowchart outlining the entire procedure of sterilizing explants:



3.4 APPLICATION OF PLANT TISSUE CULTURE

Large quantities of identical explants are produced using meristem and shoot culture of the plant for use in commercial production for potting, florist subjects, and landscaping. This aids in protecting the one unique and endangered species. It is quicker to test cells for herbicide resistance/tolerance, stress-tolerant plants using explants, tissue culture, and/or cells rather than actual plants. Large quantities of identical explants are produced using meristem and shoot culture of the plant for use in commercial production for potting, florist subjects, and landscaping. This aids in protecting the one unique and endangered species. It is quicker to test cells for herbicide resistance/tolerance, stress-tolerant plants using explants, tissue culture, and/or cells rather than actual plants. Explants are grown extensively in liquid culture in order to increase the production of recombinant proteins that are used to make

biopharmaceuticals and secondary metabolites from plants.48 This is even more helpful when related species need to be produced through the regeneration of novel hybrids and/or protoplast fusion. In order to save rare species through cross-pollination, plant tissue culture is also used. Explants are grown extensively in liquid culture to increase production of recombinant proteins that are used to make biopharmaceuticals and secondary metabolites derived from plants. This is much more helpful when it's necessary to regenerate unique hybrids or combine protoplasts to create related species. In order to save endangered species, plant tissue culture is also examined for cross-pollination with distantly related species.

Transgenic plants in agriculture

Transgenic plants are still being widely propagated for use in agriculture around the globe in order to improve agricultural production and growth potential. Plants free of disease Tissue culture enables the development and genetic homogeneity of disease-free plant material, eradicating the vulnerability of plants to pathogenic organisms. Genetic modification Due to its usefulness, one of the most popular, established methodologies, and most important tissue culture applications. Two approaches are used in the genetic transformation; one is the introduction of desired genetic features into the target organism. A biological vector may or may not be used in this technique to transport and distribute genetic data. Plants as bioreactors Transgenic plants are the source for recombinant proteins of pharmaceutical and industrial interest in a sector with the potential for innovation. Propagation and conservation Tissue culture offers an alternative for managing priceless resources like secondary metabolite levels.

Other applications

Accepted Document The soma clonal variation is a helpful technique that aids in the production of subtle variation or differentiation within species in various applications of plant cultivation. Such methods are necessary when it is necessary to expose the explants with precise doses in order to obtain the desired mutation. The control and adjustment of ploidy levels in plants can affect the expression of a variety of characteristics and enable the creation of homozygous haploid lines, genetic transformation programs, and hybridization (Scheid 1996). After fertilization, embryo rescue has the ability to play a part in genotype selection and breeding because without it, people could experience early-stage abortion. Using embryo rescue, species that have lost the ability to reproduce and prevent seed germination can be developed. Conserving genetic material makes it possible to mitigate the high rate of extinction of species and protects a nation's floral heritage[13].

CONCLUSION

Tissue culture being a superior method of plant propagation compared to the Conventional method, it promises tremendous demand in future. Plant tissue culture can therefore be an effective channel in commercializing crops, which can create jobs, earn foreign exchange and ensure a better quality of life for all. We can see that, there is huge scope of development and further research in tissue culture and it is as well very important for the ever-increasing population, and of course, India, where agriculture is still the source of income for majority. Household food and health security can be ensured through breeding of disease free, higher yielding plants, mass propagation of better-quality plants and crops with specific desirable characteristics.

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