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Review Article

OVERVIEW ON PLANT TISSUE CULTURE TECHNIQUES

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ABSTRACT:
In the area of plant science, plant tissue culture is an innovative technique which enables for certain control over a plant's growth and development by modifying its cells, tissues, and organs in vitro. An overview regarding the main methods, procedures, and applications of plant tissue culture is provided in this abstract. The initial phase in the method involves selecting explants, which are tiny plant pieces that have been sterilized to remove impurities, such leaves, stems, or embryos. After that, these explants are cultivated in a nutritional medium that contains vitamins, hormones, and other necessary components. To promote cell division, differentiation, and organogenesis, the culture conditions such as temperature, light, and humidity are maintained carefully.Plant tissue culture uses several basic techniques such somatic embryogenesis, that produces embryos from somatic cells, and micro propagation, which multiplies identical plants instantaneously. In the process of regeneration, callus induction, organogenesis, and embryogenesis are critical phases. In order to generate transgenic plants with specific characteristics, genetic modification techniques such as gene transfer and editing have also been included into plant tissue culture.Plant tissue culture has a wide range of significant applications, including the propagation of plants, preservation of germplasm, preservation of endangered species, and the development of disease-free plants. In addition, it is an efficient tool for fundamental research, enabling scientists to investigate plant physiology, biochemistry, and genetics in controlled conditions.The implementation of plant tissue culture techniques has reshaped agriculture and plant biology by providing a flexible platform for modification and development of plants. The field's ongoing progress holds great potential for tackling worldwide challenges such as sustainable agriculture, preservation of the environment, and food security.

Key words: Plant tissue culture, Organogenesis, Embryogenesis, Somatic cells, sterilized medium, controlled conditions.

INTRODUCTION

Plant tissue culture is a biotechnology method that uses aseptic conditions to grow plant cells, tissues, or organs. With this technique, tiny pieces of plant tissue called explants can be used to sterilely multiply plants. By placing the explant in a growth medium that has the essential minerals, vitamins, and hormones, scientists can encourage the development of plant tissues, which may eventually grow into the entire plant. Numerous uses for this technology can be found in horticulture, research, and agriculture.[1].Bypassing the conventional seed germination process, this makes a possibility to produce genetically identical plants, or clones, with desired traits. Plant tissue culture plays a key role in the rapid multiplication of rare or endangered species, the mass manufacturing of superior plant varieties, and the development of disease-free plants[2].The controlled conditions of plant tissue culture make it possible for studying genetics, molecular biology, and plant physiology at the cellular level. It is an essential tool in breeding projects that aim to develop crops with enhanced features, like better disease resistance and nutritional value. Plant tissue culture is a vital technique that greatly advances plant science and biotechnology by providing accuracy and repeatability in addressing issues related to agriculture, conservation of biodiversity, and scientific research.[3,4]

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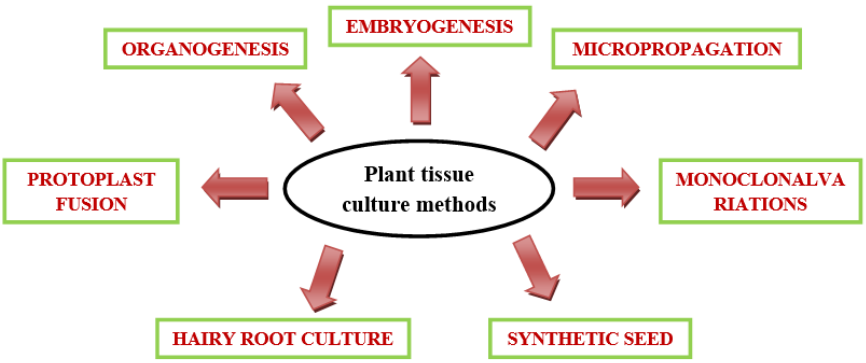

Figure.1: Plant tissue culture

ORGANOGENESIS:

The process by which new organs, like shoots and roots, evolve from cultivated plant cells or tissues is termed as organogenesis. Plant biotechnology utilizes this technique widely for modification of genes, conservation of endangered species, and mass production of plants with desired characteristics. [5]

The essential procedures and variables in organogenesis in plant tissue culture are listed below: [6, 7]

Initiation of Culture: Explants, or tiny fragments of plant tissue includes leaves, stems, or embryos, are isolated at the initial stage of the process. Then, a nourishing medium containing essential nutrients, vitamins, and plant growth regulators is used to nurture these explants.

Callus Formation: During dedifferentiation, explants often produce a cluster of undifferentiated cells named as callus. It involves cells that remain capable of growing into different plant tissues; the callus is an essential intermediate step in the organogenesis process.

Induction of Organogenesis: The level of particular plant growth regulators in the culture medium is altered in order to induce organogenesis. Auxins and cytokines are often used to regulate the direction of differentiation. For example, high cytokine concentrations and low auxin concentrations frequently encourage the growth of shoots, whereas high auxin concentrations and low cytokine concentrations induce the formation of roots.

Shoot Formation: Cells within the callus differentiate into shoot primordia, which eventually grow into new shoots, in the presence of suitable growth regulators. To create whole plantlets, the branches can be rooted and spread further.

Root Formation: In a similar manner, roots may be formed by altering the levels of auxins and cytokines that will lead to root primordia to form. Building a comprehensive and functional production facility requires this.

Acclimatization: Plantlets need to get adapted to their natural surroundings after their branches and roots begin to develop. In order to ensure their survival when shifted to soil, this involves gradually exposing them to external factors such as light, temperature, and humidity.

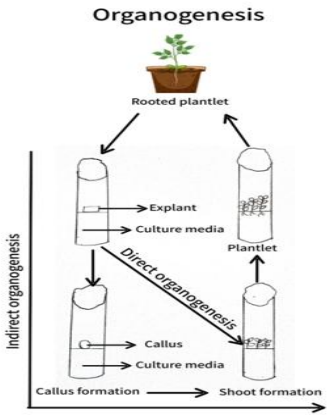

Figure.2: Organogenesis

APPLICATION:[8, 9]

Crop Improvement: To produce plants with improved traits such as disease resistance, drought tolerance, and increased yield, organogenesis is employed in crop improvement programs. Researchers can generate plants with desired traits by introducing specific genes and modifying the culture conditions.

Genetic Engineering: A crucial stage in the genetic engineering or genetic transformation of plants is called organogenesis. Transgenic plants can be created by introducing foreign genes into plant cells during tissue culture. With the use of this technology, crops with higher nutritional value and increased resistance to pests, illnesses, and herbicides have been produced.

Production of Secondary Metabolite: Some plant species yield valuable secondary metabolites with industrial or pharmaceutical uses, such as flavonoids, essential oils, and alkaloids. These secondary metabolites can be produced in culture by organogenesis, which offers a regulated setting for their extraction

Disease Elimination: Organogenesis is a viable strategy for the eradication of plant diseases caused by infections. It is feasible to produce disease-free individuals by cultivating healthy plant tissues that are free of pathogens and regenerating plan.

EMBRYOGENESIS:

In plant tissue culture, the term "embryogenesis" covers the process used to promote the growth and development of embryos from plant cells in an in vitro environment. Plant biotechnology employs this technique widely for a number of reasons, such as genetic transformation, large-scale plant production, and germplasm preservation. In a controlled environment, plant tissue culture provides the ability to alter cells and commence and conclude the process of embryogenesis.[10]

The following are the main processes in plant tissue culture embryogenesis:

The Origins of Cultures: [11, 12]

The first step in this process involves determining the explants, which are tiny fragments of plant tissue. Typically, young, actively proliferating tissues like shoot tips, embryos, or meristematic regions are used to create these explants. After surface sterilizing the explants to get rid of pollutants, they are cultivated on a nutrient medium that has specific plant growth regulators in it.

Callus Development: The explants may dedifferentiate after initiation and create a mass of undifferentiated cells termed as a callus. The growth of calluses is essential for early embryogenesis.

Embryogenic Callus Induction: Auxins and cytokines are two examples of plant growth regulators that are altered in the culture media to promote the development of embryogenic callus. These hormones affect the cells potential and leads to embryogenesis.

Embryogenesis Somatic: Instead of fusing gametes, somatic embryogenesis is the process by which embryos are formed from non-reproductive somatic cells. It involves the direct growth from the callus or other explant tissues of entities resembling zygotic embryos. The embryos move through globular, heart-shaped, torpedo-shaped, and cotyledonary phases, similar to zygotic embryos.

Development and Maturation: Somatic embryos require maturation and development after they are generated. In order to encourage the development of fully developed embryos with specific shoot and root structures that involves altering the culture conditions, such as modifying the level of nutrients and growth regulators.

Germination: The embryos are placed in a medium that promotes germination after they have matured. This is the process by which the embryos develop into branches and roots, which eventually produce plantlets.

Transition to Soil and Acclimatization: After development, the plantlets gradually adjusted to the outside conditions of the tissue culture setting. Usually, this is accomplished by gradually exposing them to external light and humidity. Plantlets can be moved to soil for further development and growth if they are strong enough.

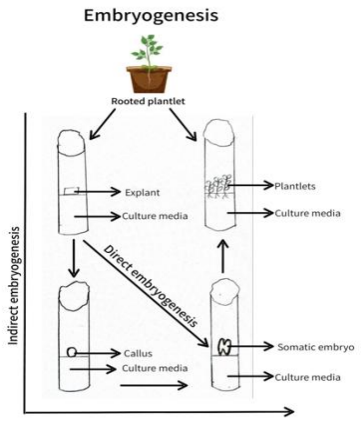

Figure.3:Embryogenesis

APPLICATION:[13, 14]

Massive Clonal Transmission: The major development of genetically identical plants is one of the primary applications for somatic embryogenesis. This is very helpful for growing plants with desirable features, high-yielding cultivars, or elite genotypes. It allows the large-scale, rapid multiplication of particular plants.

Conservation of Germplasm: In a controlled environment, somatic embryogenesis provides a way to preserve the genetic variety of plant species. It makes it possible to retain plant genetic material for a long time in the form of embryos, which may be kept in cryopreservation facilities at low temperatures.
Genetic Transformation: The genetic modification of plants frequently begins with somatic embryogenesis. By inserting foreign genes into embryogenic tissues, transgenic plants can be created with desired characteristics such increased tolerance to environmental challenges, greater nutritional content, or resistance to diseases or pests.
Elimination of Diseases: Plants in absence of disease can be grown with the help of somatic embryogenesis. It is possible to kill infections and create disease-free plants by growing cultures from healthy tissues and run via tissue culture process.

MICRO PROPAGATION:

Miro propagation is also called as tissue culture or in vitro propagation where the plant cells, organs, or tissues were cultured in an artificial nutrient medium under aseptic condition in order to produce multiple clones of the plant. This approach is very useful for rapidly growing plants with specified characteristics.[15]

An outline of the micro propagation procedure is given below:[16, 17]

The Origin of Culture: The initial stage in this process is to select an appropriate plant with the needed characteristics. Plant material, including shoot tips, nodal segments, and meristems, is collected and surface sterilized to remove contaminants.
Building an Aseptic Culture: Once the plant material has been sterilized, it is put on a nutrient medium that contains vitamins, carbohydrates, auxins, cytokines, and a variety of macro and micronutrients. The culture is kept in a controlled setting with particular humidity, light, and temperature parameters.
Shooting Expansion: Cytokines are applied carefully to the apical meristem, often referred as the shoot tip, to stimulate the production of numerous shoots. The shoots are regularly cultured onto new media in order to promote further development.
Rooting: Once developing a sufficient number of shoots, the plants are moved to a medium that contains several hormones in order to stimulate the creation of roots. Following that, rooted plantlets are exposed to outside environmental factors in order to gradually adjust them to the soil's conditions.
Transplantation: The formed roots and well-developed plantlets are prepared for transplantation into soil or other medium for growth.

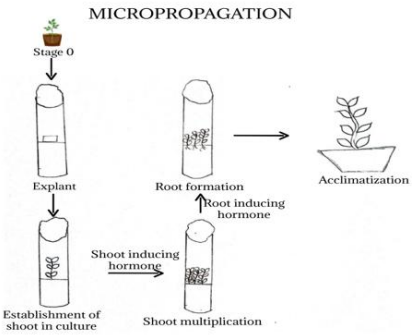

Figure 4:Micropropagation

APPLICATION:[18, 19]

Mass Clone Production: Micro propagation makes it possible to quickly and extensively produce genetically identical plants, guaranteeing consistency in characteristics like quality, yield, and resistance to disease.
Crop Improvement: Through micro propagation, agricultural crops with desired features, such increased yield, disease resistance, or tolerance to environmental stress, can be replicated on a wide scale.
Plant Material Free of Diseases: Micro propagation guarantees the generation of plant material free of diseases. This is especially crucial for crops that are vulnerable to certain diseases. Starting with a clean culture reduces the possibility of disease transmission.
Quick Plant Multiplication for Research: Micro propagation is a common technique used by researchers to swiftly produce a large number of plants for testing. This is particularly helpful for research on the physiology, genetics, and biotechnology of plants.

PROTOPLAST FUSION:

In a specialized biotechnological procedure known as "protoplast fusion," separate cells known as "protoplasts" are fused together by removing their inflexible cell walls and allowing their membranes to fuse. Protoplast fusion has three uses in the various fields of plant biology, genetics and biotechnology.[20]

An outline of the protoplast fusion procedure is given below:[21,22,23]

The selection of donor cells: Protoplast fusion has a number of applications, including genetic engineering, plant breeding, and the enhancement of microbial strains. Depending on the intended use, donor cells may

originate from a variety of sources, such as distinct plant species, varieties, or even organisms. The desired characteristics or genetic material should be present in the donor cells.

Fusion of Protoplasts: This process usually involves joining separated protoplasts together and encouraging the fusion of their cell membranes.

Various techniques can be utilized for this objective:

Chemical Fusion Agents: To promote the fusion of protoplasts, chemical fusogens like polyethylene glycol (PEG) are frequently employed. PEG has the ability to cause nearby cell membranes to fuse together, allowing their contents to combine.

Electric Fields: Protoplasts can also be fused using electric fields. The protoplasts cell membranes are temporarily made permeable by an electric pulse, which enables them to fuse when they come into close proximity.

Biological Fusion: Protoplast fusion can occasionally be aided by biological agents such as viruses.

Regeneration: The resulting cells from the fusion process can be developed and regenerated to produce whole animals. Regeneration circumstances are determined by the cell type and species. Specific culture media, growth regulators, and regulated ambient conditions are some of the conditions that may apply to plants.

Selection and Characterization: The resulting fused cells or organisms must be carefully chosen and characterized in order to guarantee that the intended traits or genetic combinations are present. This could entail performing genetic analyses, looking for particular markers, or assessing the phenotypic traits of the regenerated organisms.

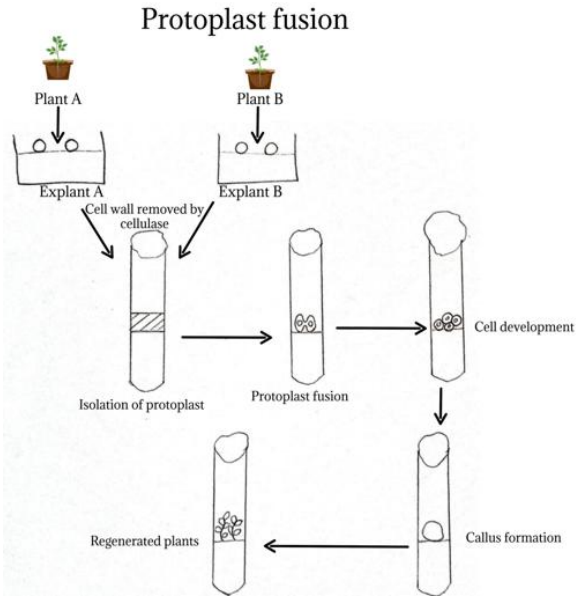

Figure.5:Protoplast fusion

APPLICATIONS:[25, 26]

Hybrid Plant Development: The process of protoplast fusion is frequently employed to create hybrid plants that blend desired characteristics from two distinct parent plants. This may result in the development of novel cultivars with enhanced traits like greater resistance to disease, increased yield, or enhanced ability to adapt to particular environmental conditions.

Genetic Improvement: Genetic diversity can be introduced into plant populations through protoplast fusion, which is advantageous for breeding initiatives. By utilizing this diversity, new varieties with improved genetic stability and desired traits can be created.

Production of Haploids: The process of protoplast fusion is used to create haploid plants, which have a single set of chromosomes. These haploids may be useful in breeding initiatives and genetic research.

Somatic Hybridization: Plant species can transfer certain genes or traits by means of somatic hybridization through protoplast fusion. This can be especially helpful in situations where genetic barriers make sexual hybridization difficult or impossible.

MONOCLONAL VARIATION:

"Monoclonal variation" in plant tissue culture denotes the presence of genetic variation among plants that have been generated from a single, genetically homogeneous source, usually by means of techniques such as somatic embryogenesis or micro propagation[27].

An outline of the monoclonal variation procedure is given below:

Choosing a suitable explant: From the intended parent plant, such as a tiny segment of leaf, stem, or shoot tip. The explant is sterilized to get rid of impurities.

Cultivation of the explant in a medium rich in nutrients and containing regulators of plant growth. Multiple shoots and roots from the explant are stimulated, resulting in the formation of plantlets[28].
For continued development, move these plantlets to soil or another growing medium.
Genetic Uniformity: Since plantlets generated through micro propagation are made from a single tissue or cell, they are clones of the parent plant. Because they share all of the parent plant's genetic characteristics, whether favorable or unfavorable, these plantlets display monoclonal variation[29].
Quality Control: Plant tissue culture requires quality control methods in order to ensure the genetic homogeneity of plants that have been propagated. Keeping sterile conditions, choosing and regenerating explants according to the right procedures, and routinely checking for contaminants are a few of them[30]

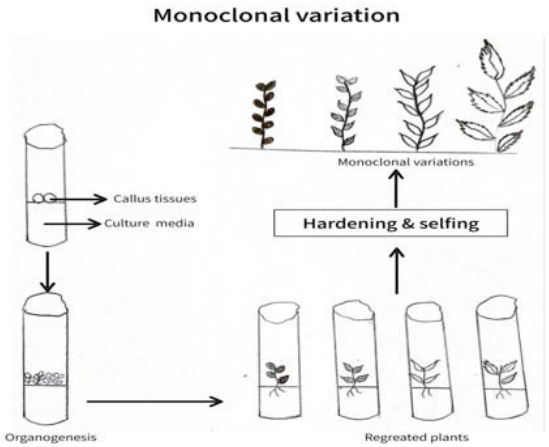

Figure.6:Monoclonal variation

APPLICATONS:
Crop Improvement: The creation of new crop varieties with enhanced traits can result from monoclonal variation. To develop new, improved plant lines, mutations resulting in traits like drought tolerance, disease resistance, or increased yield can be chosen and propagated[31].
Pharmaceutical and Medicinal Plants: Certain plant species are grown for their potential use in pharmaceuticals or other medical treatments. These plants can produce more bioactive compounds when monoclonal variation is used, which will increase the yields of important medicinal compounds[32].
Research and Education: Plant genetic and epigenetic modifications can be studied with great success due to monoclonal variation. These variations provide a useful tool for studying the molecular mechanisms underlying these modifications and their effects on plant development[33].
HAIRY ROOT CULTURE:
The technique of hairy root culture is a type of plant tissue culture in which plant roots with a thick layer of tiny, hair-like structures on their surface are induced and grown. Agro bacterium rhizogenes, a soil bacterium that can insert a portion of its DNA (the RI plasmid) into the genome of the host plant, is usually responsible for transforming plant tissues into these hairy roots. The result of this genetic transfer is the formation of the distinctive hairy roots, which are separate from regular plant roots in terms of both growth traits and outward appearance[34].
PROCESS:
Selection of parent plant: To identify your intended use, pick a plant species or cultivar that possesses the desired characteristics or the ability to generate a particular set of secondary metabolites[35].
Explant Sterilization: Plant explants, such as segments of leaves, stems, or roots, should be prepared and sterilized to remove any impurities[36].
Inoculation with Agro bacterium: Prepare the cultures of Agro bacterium rhizogenes, which are carriers of the plasmid Ri(root-inducing).The genes responsible for inducing hairy roots are present in this plasmid[37].
Apply the Agro bacterium suspension to the sterile plant explants. Injections, immersions, and other suitable techniques can all be used to cause infection[37].
Co-Cultivation: Under particular conditions, incubate Agro bacterium infected plant explants. Under particular conditions, incubate Agro bacterium infected plant explants[38]
Selection and Regeneration: Plant explants should be transferred to selective media containing antibiotics or herbicides following co-cultivation. These medicines kill the Agrobacterium while allowing altered plant cells to regenerate. After a few weeks, the changed tissues begin to reveal hairy roots.[39].

Subculture and Development: Remove the emerging hairy roots and place them in new culture media to encourage growth and multiplication. The morphology of hairy roots is characterized by fine branching[40].
Maintenance: Hairy root cultures can be kept in liquid or solid media and are kept under sterile conditions. In order to maintain their growth, a portion of the hairy roots are routinely transferred to new media during subculture[41].
Detailed Description: Thepresence of the Ri plasmid and particular genetic markers allows for the characterization of the genetically altered hairy root cultures. This contributes to confirming the transformation's success[42].
Bioreactor Cultivation: Hairy root cultures can be moved to bioreactors for larger-scale production, where exact control over parameters like aeration, pH, and nutrient supply is possible for optimal growth and metabolite production[43].
Harvesting and Extracting Compounds: Once the desired secondary metabolites have accumulated to a sufficient amount, the hairy roots are harvested and processed to extract the target compounds for additional uses[44].

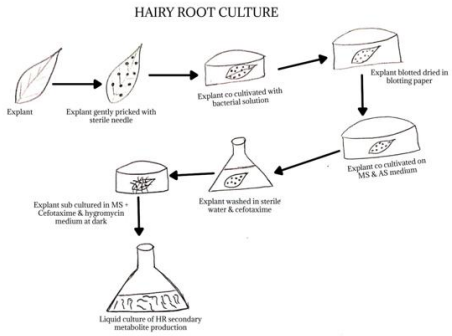

Figure.7: Hair Root Culture

APPLICATIONS:
Pharmaceutical Industry: Taxol and ginsenosides are two examples of the phytochemicals and medicinal compounds that are produced using hairy root cultures for use in pharmaceutical products[45].
Agriculture: They work on creating crops that are resistant to disease and studying the interactions between plants and pathogens[46].
Research: Hairy root cultures are useful resources that help scientists investigate different aspects of plant biology and genetics[47].
Environmental monitoring: Hairy root cultures are a useful tool for evaluating the ways in environmental stresses, pollutants, and heavy metals affect the physiology and growth of plants[48].
SYNTHETIC SEED TECHNIQUE:
The synthetic seed technique is a method in plant tissue culture that involves encapsulating somatic embryos, shoot buds, or other embryogenic structures in a protective covering to create structures resembling seeds.Plant material can thereafter be propagated, stored, and transported using these "synthetic seeds". The conservation of genetic resources and the mass manufacture of plants with desired features are two areas in which the synthetic seed approach excels[49].
The crucial actions and factors in the synthetic seed technology are as follows:
Selecting Explants:
Choosing appropriate explants is the first step in the procedure; they can be shoot buds, somatic embryos, or other embryogenic structures obtained via tissue culture.
Encapsulation: A gel-like matrix made of a combination of nutrients, gelling agents (such sodium alginate), and other additives encapsulates the selected explants. The emerging plant structures are shielded by this encapsulation[50].
Gel Formation in Droplets: Typically, the encapsulated explants are dropped one by one into a solution containing divalent cations, such as calcium nitrate or chloride. The encapsulation matrix gels due to the divalent cations, enveloping the explant in a layer of defense[51].
Hardening: After that, the explants that were enclosed in gel are left to harden and form structures that resemble seeds. In order to surround the plant material with a protective barrier, this hardening stage is crucial[52].
Maturation and Development: To enable additional development and maturation, the synthetic seeds are cultivated in vitro under carefully monitored circumstances. In order to support the growth of shoots and roots from the enclosed structures, the proper nutritional media and growth regulators must be provided[53].
Storage: If the right circumstances are met, synthetic seeds can be kept for a long time. Long-distance plant material transportation and the preservation of plant germplasm both benefit from this storage capability[54].

Germination: When it's time to start growing, the artificial seeds can be germination-started by taking off the gel coating and putting them in an appropriate germination medium. As a result, seedlings with roots and shoots begin to form[55].

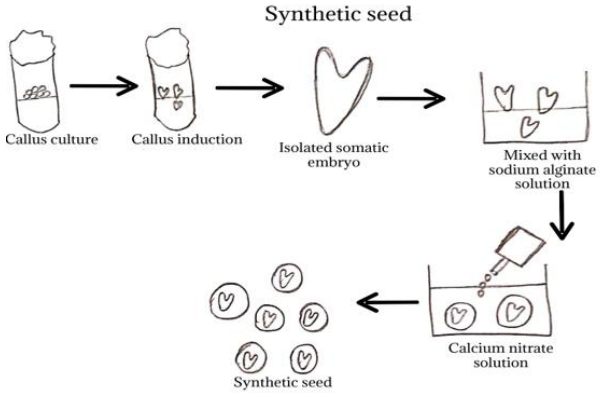

Figure.8:Synthetic Seed

APPLICATION:

Uniformity: Large-scale agricultural output benefits from the great degree of size and genetic composition uniformity that synthetic seeds provide[56].

Storage and Transportation: Plant material may be more easily stored, transported, and distributed over great distances thanks to the encapsulation, which protects the plant material[57].

Fast Multiplication: Plants with desirable features can be multiplied quickly thanks to the synthetic seed technology, which speeds up breeding initiatives[58]

Illness-Free Propagation: Since the encapsulation process helps get rid of possible pathogens, synthetic seeds can help produce plants free from illness by starting with healthy explants[59].

Genetic Resource Conservation: By giving plant species that are uncommon, endangered, or important a way to be stored and propagated in a controlled setting, synthetic seeds can help protect genetic resources[60].

CONCLUSION:

Therefore, plant tissue culture is a powerful and adaptable method in plant biotechnology, which enables rapid propagation, conservation of rare species, and production of disease- free plants. It is useful in span agriculture, horticulture, and pharmaceutical industries, offering solutions for food security and sustainable crop improvement. This technique is also used in genetic engineering and secondary metabolite production. Despite problems like as pollution and expensive costs, technological and procedural innovations continue to improve its efficiency and accessibility. Overall, plant tissue culture is critical for improving plant science and fulfilling the rising demands of global agriculture and biodiversity conservation.

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