Chapter IV: In Vitro Multiplication Techniques

1- Definitions

a) Biotechnology is a set of biological techniques applied for the production of biomass from animal or plant cells.

b) Plant biotechnologies are industrial biotechnologies where plant material is the raw material.

c) Plant biotechnologies use plant tissue culture to improve agricultural or industrial production. They allow for the improvement and production of plants.

- The main goals of plant biotechnologies are:

1 - The massive multiplication of healthy and/or hybrid plants, potentially for commercial production.

- The creation of new genotypes through

3- Selection of interesting somaclonal or gametoclonal variants exhibiting resistance to biotic or abiotic stresses.

4 - Haplomethods (gametes)

5 - Protoplast fusion

6 - Genetic transformation

7 The use of plant cells for the massive production of high-value molecules (secondary metabolites):

2. Tissue Culture and Organ Regeneration

In a tissue culture, the stages leading to organ regeneration are comparable to those observed during cutting propagation. A phase of dedifferentiation and the resumption of mitotic activity precedes the organogenic phase; the latter occurs either through the immediate establishment of a program leading to the direct morphogenesis of stems or roots (Fig. 1), or through the formation of a disorganized cell mass, the callus, within which organogenic competence appears later (Fig. 2).

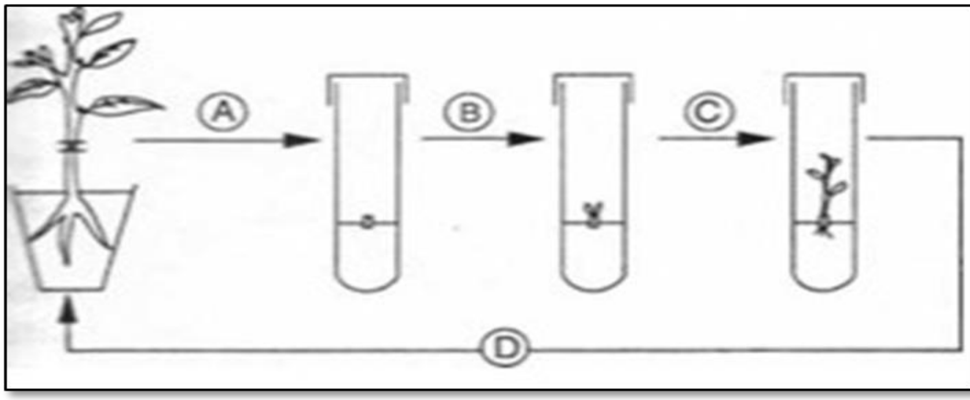


Figure 1: Vegetative multiplication by regeneration of stems and roots on explants.

A) Primary explant culture initiation; B) Neoformation of buds which develop into stems on a medium with a high cytokinin/auxin ratio; C) Root regeneration after transfer to a medium with a low cytokinin/auxin ratio; D) Acclimatization to soil and transplantation to a pot.:

The neoformation of buds begins with the resumption of mitotic activity in differentiated cells, which, by undergoing repeated divisions, constitute meristematic masses; within these masses, the orientation of the divisions allows for the organization of shoot apical meristems (Fig2).

The development of these buds leads to the formation of leafy shoots. The fragments bearing the neoformed shoots can then be transferred onto a new medium in which the cytokinin/auxin balance favors auxin, to ensure rooting.

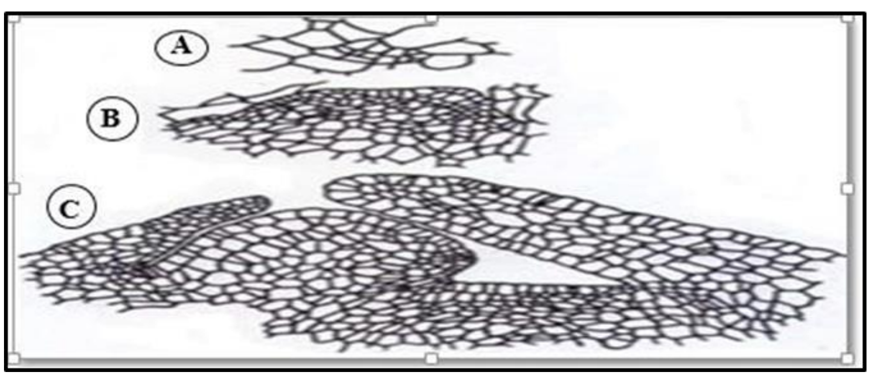


Figure 2 Neoformation of a bud in a Chicory tuber explant.

A) Early cell divisions of parenchyma cells;

B) Organization of a shoot apical meristem following oriented mitoses; the epidermal layer is differentiated.

C) Longitudinal section of a fully formed meristem with leaf primordia.

The neoformation of adventitious roots is often achieved in the presence of IAA (Auxin). As with adventitious buds, it requires mitotic reactivation that leads to the organization of a typical root meristem.

I-Meristem Culture

Meristem culture particularly involves the cultivation of the shoot apical meristem. This technique is also called a shoot tip or apical meristem culture. It was reported in the year 1952 by the scientist's named Morel and Martin. Orchid Cymbidium was micropropagated using this culture by Morel in 1965.

The meristem culture is very similar to the technique of micropropagation, which also involves:

1 - Initiation of culture
2- Multiplication of culture
3- Rooting of a developed shoot
4- Transfer of plantlets to the pots

For the regeneration of plant, Murashige and Skoog's (MS) medium is prevalently used with low salt concentration for the majority of the species. Fungicides (Bavistin) or antibiotics (chloramphenicol or streptomycin) is also used to remove endophytic contamination. You will get to know the definition, process, uses, advantages, and limitations of the meristem culture method in this context.

Definition of Meristem Culture

Meristem culture is defined as the tissue culture technique, which uses apical meristem with 1-3 leaf primordia to prepare clones of a plant by vegetative propagation. This technique primarily involves the isolation of the meristem by applying a V-shaped cut in the stem. By culturing the shoot meristem, adventitious roots can be regenerated in this method. The shoot tip's preferred size is 10 mm for the generation of the virus-free plant, but the shoot tip's size does not matter in vegetative propagation.

What is Meristem?

Meristem is a part of the plant, which plays a key function to increase the plant length. It possesses meristematic cells that are continuous, oval, polygonal and rectangle in shape. There is no intercellular space between the meristematic cells.

Structure of meristem

Meristem is the shoot tip, which is found at the shoot apical and root apical region. It is dome-shaped, measuring approximately 0.1 mm in width and 0.25-0.3 mm in length. The apical shoot meristem is the portion where a stem elongates and is free of pathogens proved in 1949 before introducing meristem culture.

Limmaset and Cornuet were the two scientists who observed the declined growth of virus towards the apical meristem. Thus, meristem culture is prevalent among other techniques in producing plants free from virus and other related organisms.

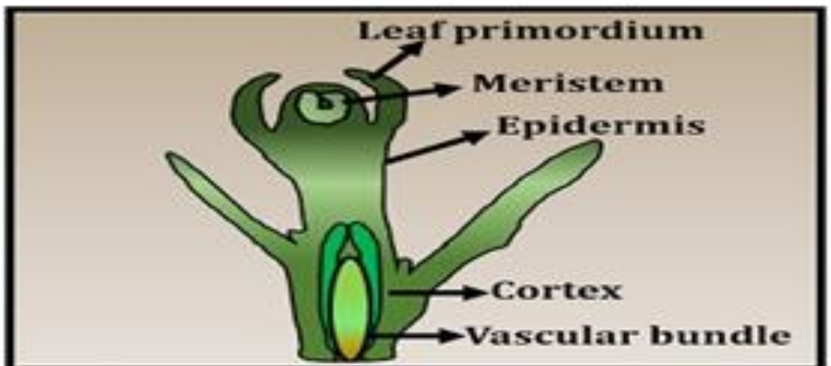


DIAGRAM SHOWING MERISTEM

Process of Meristem Culture/ It involves the following steps:

Protocol of meristem culture;

1- Remove the young twigs from the healthy plant.

2- Cut the tip portion of the twig that should be of 1 cm.

3- Subject the shoot tip to the surface sterilization in the sodium hypochlorite solution for 10 minutes.

4- Rinse the explants thoroughly with the distilled water for about 4 times.

5- Then, transfer each explant to a sterilized Petri plate.

6- Dissect the outer leaves of the shoot tip.

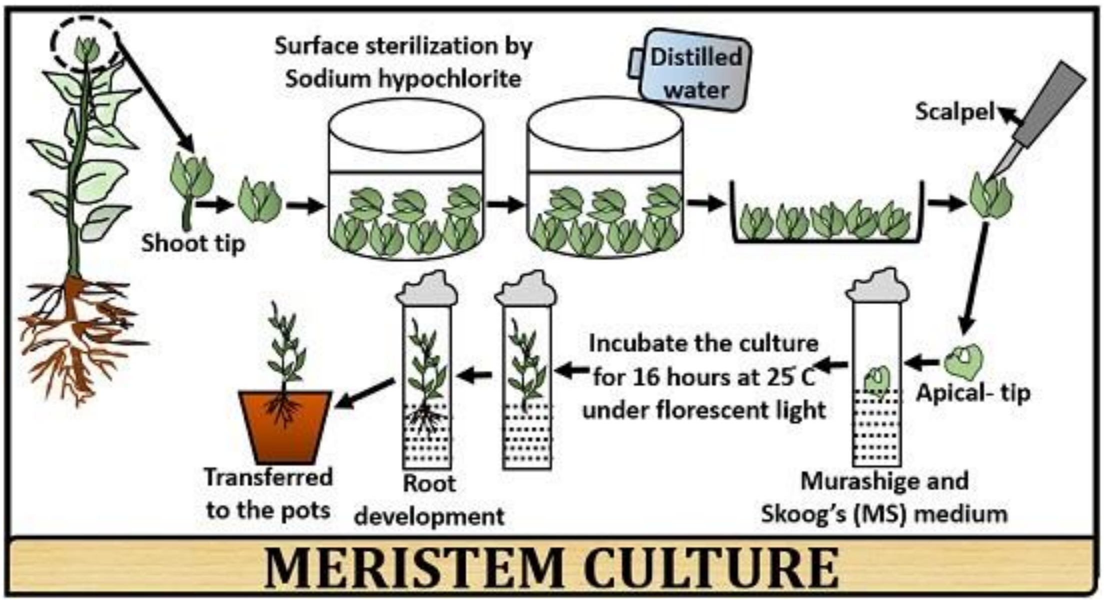
7- After the dissection of outer leaves, the apex region will be exposed and separated using a sharp scalpel.

Transfer the apex or apical meristem to the MS (Murashige and Skoog's) nutrient medium.

Incubate the culture under the light at 25 degrees C for 16 hours.

After the development of a single or multiple shoots, transfer it to the hormone-free medium for the root development.

Then, transfer the plants to the pots filled compost and keep it under greenhouse condition for hardening.



Applications

By the Meristem culture, virus-free plants can be produced. The plant meristem that produces heterozygous seeds can be stored in the in-vitro conditions.

It also helps in the plant breeding technique, in which the hybrid breeds of the plants can be grown. The quarantine authority for the international exchange easily accepts the plants obtained by the meristem culture.

It is also useful in the micropropagation technique, which involves the vegetative or asexual propagation of the whole plant.

Advantages

1- Apical shoot culture also helps in the production of virus-free plants.

2- The germplasm or the seeds can be conserved in-vitro or by the cryopreservation method.

3- Meristem contains high auxin concentration that promotes plant growth.

Disadvantages

1-Isolation of meristem is quite difficult.

2- Explants take more regeneration time to grow.

II- Protoplast Culture: Isolation and Culture Methods

Tissue culture is an advanced biotech technique used in labs to grow disease-free plants on a commercial scale. And, among the many tissue culture techniques, such as callus culture, organ culture, and embryo culture, one is the protoplast culture technique.

In protoplast culture, protoplasts isolated from any plant part, including root, shoot, leaves, or embryo, is cultured in an artificial media under artificial conditions favoring cell division and plant regeneration.

In the previous part of this article, you've learned what is protoplast culture, how protoplasts are isolated from the plant parts, and how their viability is checked for their use in tissue culture procedures.

In this article, we will review the types of culture media types used to grow protoplasts in a lab environment and the techniques that are used by scientists to culture protoplasts.

Protoplast Culture Media Types

The culture medium contains all the nutrients and vitamins required for the growth and development of plants in lab conditions. Mostly MS media is used for such purposes. However, often a modified MS media or B5 media is found to be suitable for the regeneration of plants. It means the formulation of the culture media depends on the species of the plants and their specific growth requirements.

Some special feature of protoplast culture media are given below:

There should be less iron and zinc and no ammonia in the culture medium.

For the membrane stability, the calcium concentration should be 2-4 times more compared to that used in normal cell culture media.

Mainly glucose is the preferred source of carbon, however, a combination of glucose and sucrose can also be used.

A high auxin/kinetin ratio is required to induce cell divisions, however, a high kinetin/auxin ratio is needed for regeneration.

Some vitamins can be used as they are used in standard tissue culture media.

Other than the nutritional components, the osmoticum (the chemical which increases the osmotic pressure of the solution) is another factor that needs to be taken care of while preparing the tissue culture media.

For plant regeneration from protoplasts, first, they are required to develop cell walls to proceed to the stage of cell division. Protoplast is cultured either on semi-solid media or liquid media. However, there are also instances where protoplasts are first cultured on liquid media and then transferred to solid media for further development. **Agar Culture**

Agar is the most widely used solidifying agent in labs. As much concentration of agar should be used during the experiments that it forms a soft layer of gel after being mixed with protoplast suspension.

The protoplasts are cultured on the plate using Bergmann's cell plating technique. Here, the protoplasts are fixed in a position for cell division and further growth. The agar technique is efficient in preventing the formation of clumps in the cultures.

Liquid culture

It's the most preferred method for the protoplast culture. Using this technique, the density of the protoplasts can be manipulated and they are easy to dilute and transfer. Moreover, the osmotic pressure of the cultures can also be diluted.

The protoplasts are cultured in labs using the following techniques:

Feed Layer Technique. In this technique, protoplast suspension is exposed to X-rays and then plated on agar plates. It's used to culture protoplasts at low density. Moreover, the feel layer technique is also suitable for the selection of hybrid cells and specific mutants on plates.

Co-culture Protoplasts This technique is used to culture together protoplasts of two different species. And, you must also note that it can only be used if the protoplasts of the two plant species are morphologically distinct.

Micro-Drop Culture In this technique, protoplasts are cultures in a special dish, known as Cuprak dishes. The dish has outer and inner chambers. The inner chamber has several walls, in which individual protoplast droplets in nutrient media can be added. The outer chamber contains water to maintain the humidity for the growth of the cultures.

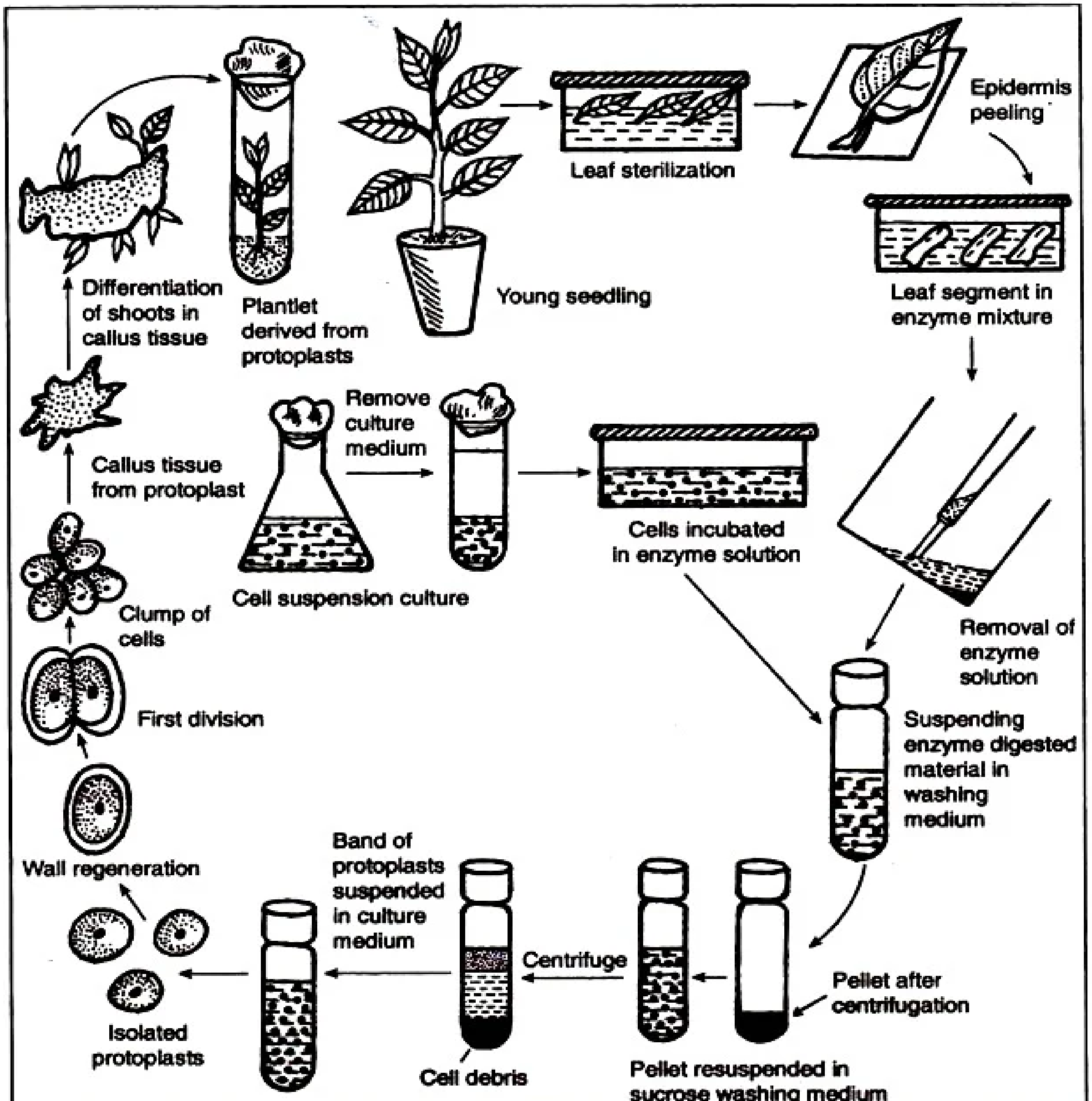


Fig. 20.3: Steps involved in protoplast isolation, culture and regeneration

Figure1: A detailed schematic diagram of the process of protoplast cultur

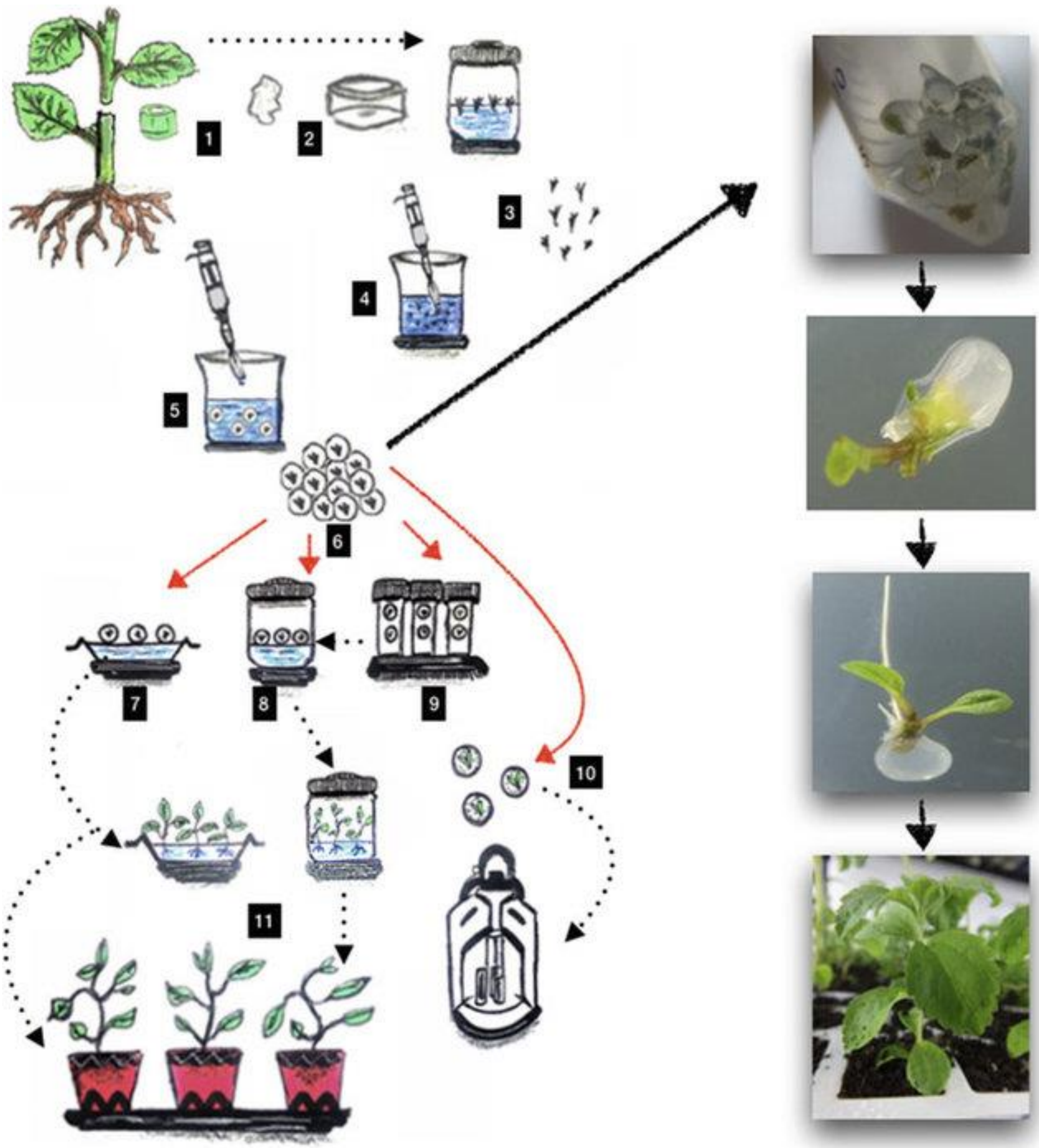
III-Artificial seeds

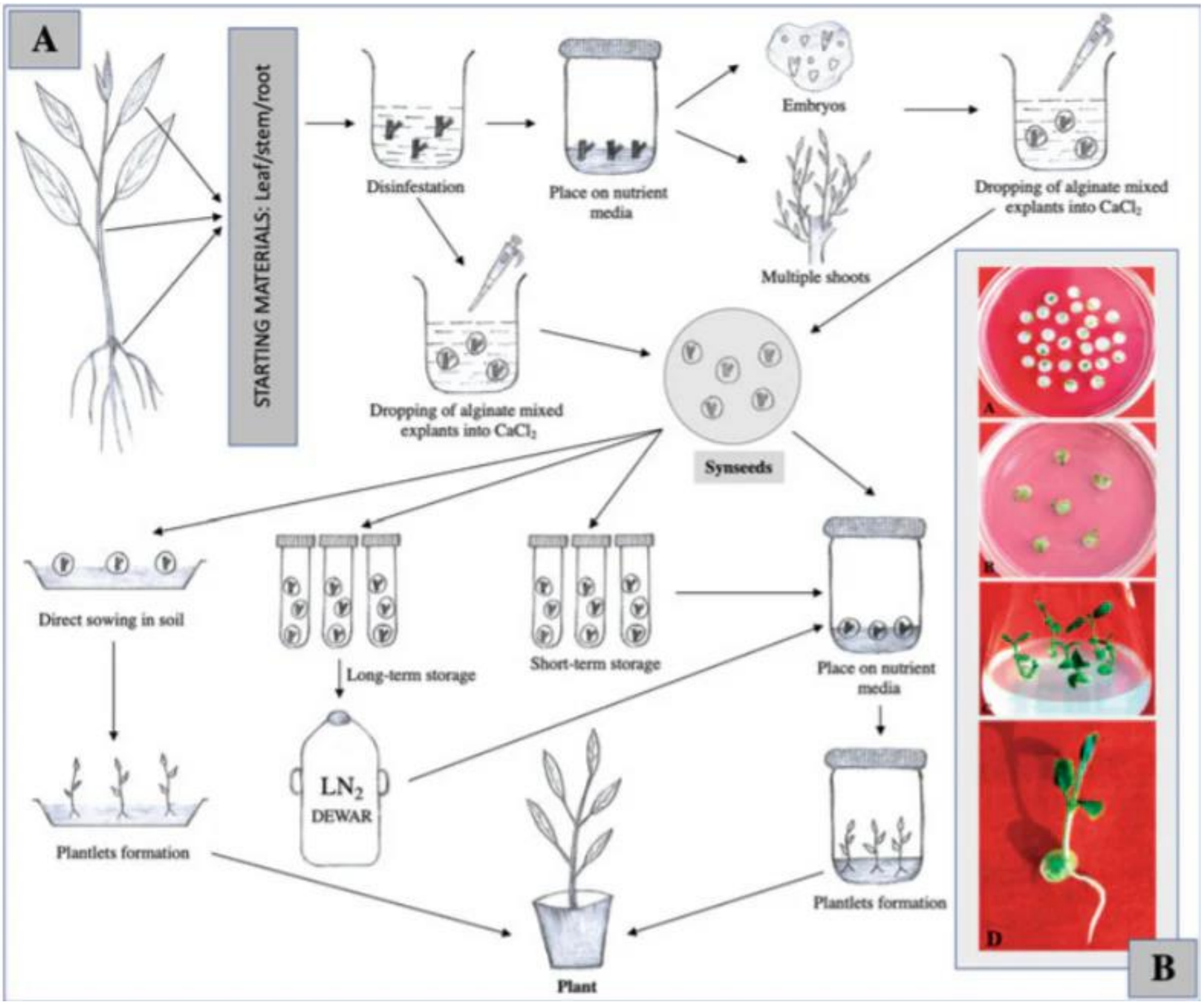
1. Selection of Plant Materials

The synthetic seeds can be produced by using somatic embryos or other plant propagules. However, a specific plant species supports a specific plant material for the production of synthetic seeds.

For example, somatic embryos have been successfully used to produce synseeds in several plant species that include Oryza sativa, Dalbergia sissoo, Curcuma amada, Hemidesmus indicus, etc.

Whereas some plants support the production of synseeds when nodal segments are used. It includes Eclipta alba, Cannabis sativa, Solanum tuberosum, Gossypium hirsutum, etc.


1
2
3
4
5
6
7
8
9
10
11



2. Selection of Encapsulation Matrix

The encapsulation of seeds is an essential factor that determines the production of synseeds. So, there are some qualities that the encapsulating material should have if it's going to be used for the production of synseeds. It includes:

1- It must not damage the embryo.

2- The coating should be mild to protect the embryo.

3-The coating should be durable for rough handling during manufacture, storage, transportation, and planting.

4- The coat must contain all the essential growth materials such as nutrients and growth regulators.

5- The formed synseeds should be transplantable using the existing techniques.

The most widely used encapsulating material is sodium alginate. However, there are some other agents as well that are used with sodium alginate for encapsulation. It includes gelatin, potassium alginate, sodium pectate, and carrageenan.

The advantages of synseed production are given below:

1- Easy handling.

2- Short- and long-term storage capacity.

3- Genetic uniformity.

4- Low-cost quality plant materials are required.

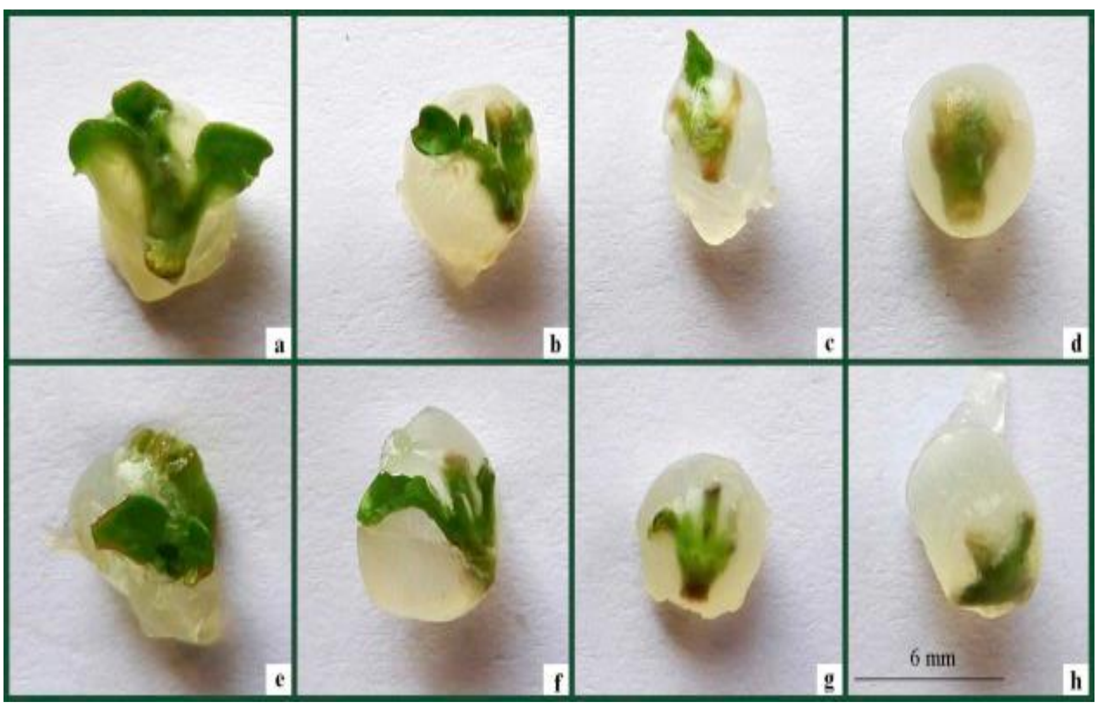
5- It allows the transportation and exchange of germplasm between national and international laboratories.

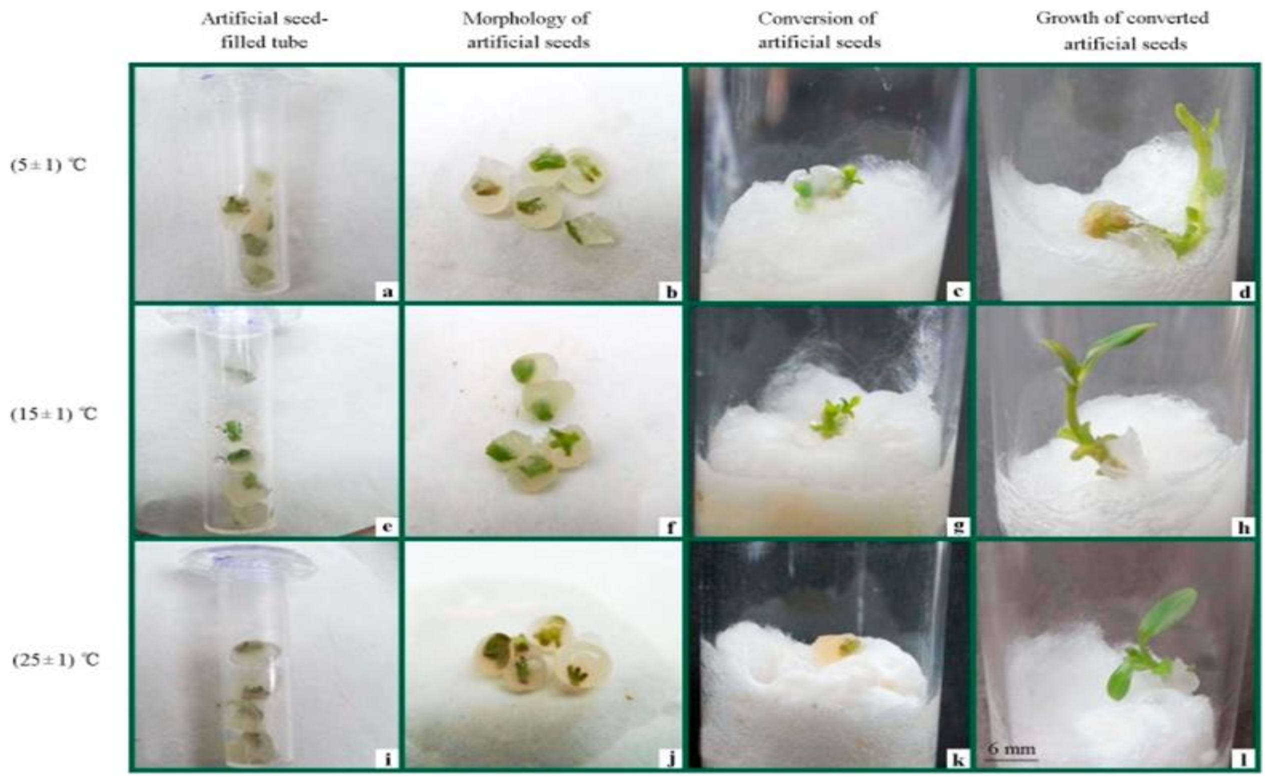
Application

The production of synseeds facilitates the growth of several plants that have low seed viability, seedless fruit, and poor germination rates, and that depend on mycorrhizal fungal symbiosis for germination.

It is very useful when it comes to genotype selection, germplasm preservation, and in vitro propagation of endangered, rare, and commercially important plants.

It also allows the conservation of plant species through short- and medium-term preservation.





References

1- Faisal, M., & Alatar, A. A. (Eds.). (2019). Synthetic Seeds. DOI:10.1007/978-3-030-24631-0

2- Bhojwani S. S. and Razdan M. K. (1983). Plant Tissue Culture: Theory and Practice. Elsevier publications.

3- Rihan Z. Hail, Kareem Fakhriya, El-Mahrouk E. Mohammed, and Fuller P. Michael (2017). 4- Artificial Seeds (Principle, Aspects, and Applications). Agronomy, 7(4), 71. DOI: https://doi.org/10.3390/agronomy7040071.

5- http://barc.gov.in/publications/nl/2000/200009-02....

6- https://www.biotecharticles.com/Agriculture-Articl...

7- https://shodhganga.inflibnet.ac.in/bitstream/10603...