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Synthetic Seeds and Its Application

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Synthetic seeds also known as artificial seeds, are encapsulated plant tissues, such as shoot buds, axillary buds, somatic embryos, shoot tips, cell aggregates or any other tissues that can be cultured as a seed and grown into a plant under either in vitro or ex vitro conditions and have the potential to maintain their viability after cold storage. Previously, artificial seeds were produced by encapsulation of the somatic embryo; however, in recent years, synseeds have been produced by the encapsulation of various in vitro-derived propagules such as nodal segments containing axillary buds, apical shoot buds and stem. Murashige (1977) was the first researcher to discuss the concept of artificial seeds, while desiccated artificial carrot seeds were first produced by Kitto and Janick (1982). Later, Redenbaugh *et al.* (1984) successfully developed a method for synseed production by encapsulation of somatic embryos of alfalfa in sodium alginate.

Selection of propagating material: Selection of planting material is the important key for successful development of synseeds. There were different types of planting material can be used which are discussed below.

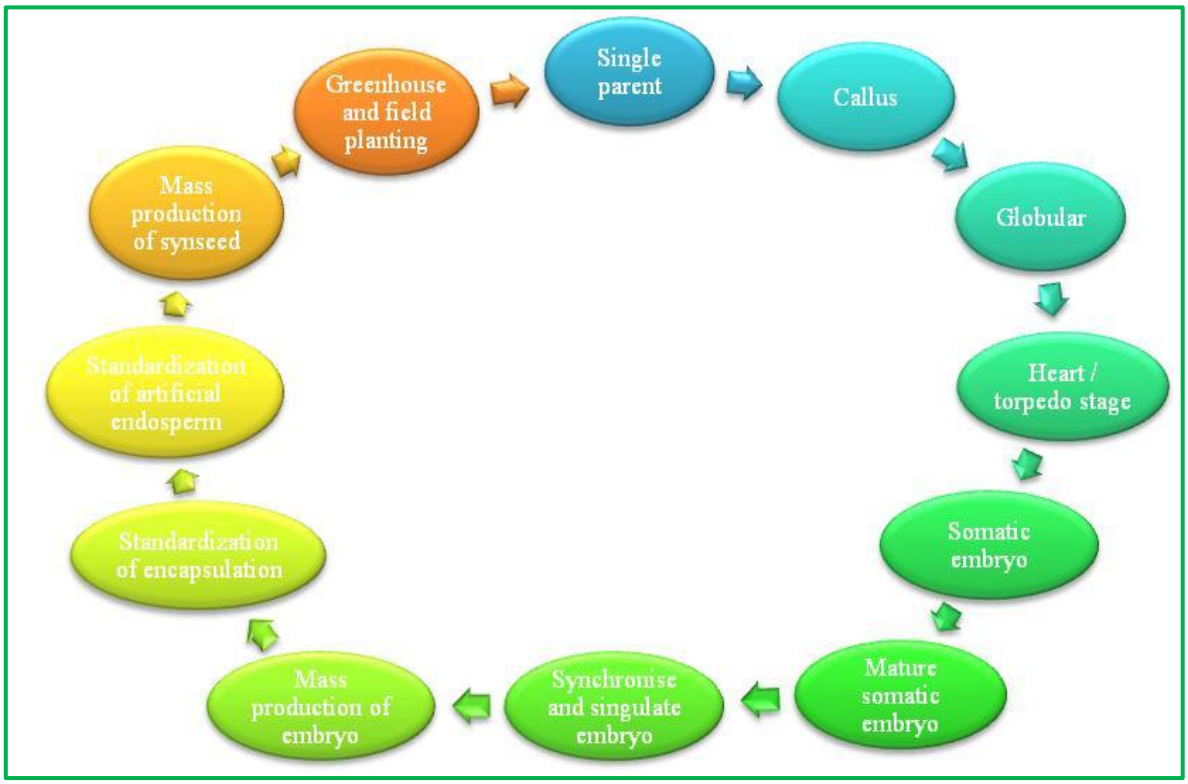
1. **Somatic embryo**: Bipolar structures that contain both the shoot and root poles are described as somatic embryos. These are the most suitable material for synseed seed production because of their polar nature, which means they are able to develop roots and shoots in a single.
2. **Nodal Segment and Shoot Tips:** The most common propagules employed for the development of synseed are nodal segments with axillary bud (micro-cuttings). This is most likely because these explants are relatively simple to produce once the micro-propagation system is established and because they have the capacity to maintain viability in terms of sprouting and conversion potential even after a substantial period of storage, which is necessary for germplasm.
3. **Callus and protocorm-like bodies:** Generally, calluses are not often used in production of synseeds. This could be attributed to the undifferentiated nature of calluses, which have several requirements for successful differentiation that limits the utility and acceptability of the use of calluses in the production of synseeds.

Selection of the encapsulation matrix: The encapsulation material is considered to be a critical factor for the production of uniform synseeds. The encapsulation material should be consistent enough to allow seed handling without breakage, but weak enough to allow the bud to break free from the capsule upon regrowth. This balance between synseed hardness and softness can be achieved by encapsulating explants with sodium alginate hydrogel. Sodium alginate is the most commonly used substance for encapsulation of explants; however, there are other agents such as sodium alginate with gelatin, potassium alginate, sodium pectate and carrageenan that are used for encapsulation. In general, sodium alginate has been shown to be the most commonly used for encapsulation because of its useful

thickness, low cost, fast gelation and nontoxic nature. It can also provide better protection for the covered explants against mechanical damage. The strength of encapsulated beads depends mainly on the concentration of sodium alginate and calcium chloride, as well as the mixing duration; however, it may vary for different explants and plant species. Furthermore, the addition of nutrients and growth regulators to the encapsulation matrix is also an important factor for successful synseed production, as it increases the reliability of germination and the viability of the synseeds. These matrices are considered to be artificial endosperms and they also play an important role in the storage of synseeds at low temperatures and in regrowth ability after transfer to germination media.

Method

1. **Encapsulation Matrix:** The required concentrations of sodium alginate solution (0.5–5.0% w/v) were prepared in liquid nutrient medium or double distilled water to form the gel matrix. Similarly, calcium chloride solutions were prepared at different concentrations (25–200 mM) in double distilled water to form the complexing agent. Both the gel matrix and complexing agent were autoclaved at 121°C and 1.1 kg cm^{-2} pressure for 15 min.



2. **Encapsulation:** After preparation of gel matrix and complexing agent, selected plant materials (explants) were prepared for encapsulation as follows:
   i. The propagules were dipped in 3% sodium alginate solution.
   ii. The mixture (propagules contained within sodium alginate) was placed into calcium chloride solution (100 mM) and left for 30–40 min to allow the alginate beads to harden, forming calcium alginate around the propagules.
   iii. Calcium alginate beads were washed with sterile double distilled water two to three times to remove traces of calcium chloride.
   iv. Synseeds were transferred to sterile filter paper and left for 5 min under the laminar air flow hood to dry.
   v. The synseeds were then ready and could be stored at 4, 15 or 24°C, depending on the intended use.

Applications of Synseeds: Synseeds have several applications in different fields of plant biotechnology and the conservation of rare or endangered plant species. These applications include

- Propagation of encapsulated explants are characterized by regrowth and conversion abilities after encapsulation and storage at low temperatures, when transferred to the germination.
- Synseeds could be used for propagation and multiplication of rare and endangered plants, elite genotypes, seedless plants, medicinal plants, genetically engineered (modified) plants and commercially important plants.
- Short and medium-term conservation synseed technology offers strategies for the conservation of plant species through short and medium-term preservation. These processes are generally known as slow growth techniques. Appropriate storage conditions and a finite storage period are the most critical factors to maintain synseed viability during transportation and conservation and these may lead to the successful commercialization of this technique.
- Synseeds can be efficiently cultivated in vitro, either on semi-solid culture medium or planting substrate (e.g., perlite, vermicompost, vermiculite, soil, soilrite, sand, or gravel) for conversion into complete plantlets.
- Synseed production technology could be useful for the transportation and exchange of elite germplasms, axenic plant material and genetically engineered plants between laboratories at national and international levels for propagation, genetic engineering, breeding and pharmaceutical purposes.

Limitations

- The large-scale production of synseeds with high regeneration ability in a cost-effective manner is the first step that needs to be achieved to allow commercialization of this technique.
- The most suitable plant material for production of synseeds is somatic embryos. However, their utilization as synseeds is limited by asynchronous development, the loss of embryogenic potential with aging cultures, precocious germination, the lack of tolerance to desiccation and structural anomalies.

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