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Perspectives and Prospects of Artificial Seed Production in Crop Plants

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Abstract

Advancements in plant cell tissue culture have helped to address the problems faced with several economically important plant species. Micropropagation and encapsulation technologies have been combined to make a new innovation called 'artificial seeds' or 'synthetic seeds' or "synseed" which combines both advantages of these two technologies. Alginate coats are used for artificial seeds to encapsulate somatic embryos/vegetative buds/ micro propagules that can act as seeds. They can then be germinated into plantlets upon incubation under in vitro or in vivo conditions and retain their regeneration potential after storage at low temperatures. Germplasm preservation and exchange between national and international laboratories may rely on using encapsulated propagules of elite plants. Also, this technology has been successfully exploited for cryopreservation by use of encapsulation - dehydration, or encapsulation - vitrification in germplasm storage of elite plant species. This review gives an overview of latest developments on synseed technology with special emphasis on explant selection for making successful synseeds as well as matrices that are used as an encapsulation material for synseeds. Moreover, the constraints impeding the advancement of seed technology and related future perspectives are also discussed

Keywords: Artificial seeds, Conservation, Elite species, Germplasm, Propagation, Synseeds

INTRODUCTION

Plants reproduce through two major reproductive methods: asexual and sexual. Asexual reproduction involves vegetative propagation such as runners, tubers, and cuttings to generate new plants without seeds, while sexual reproduction involves pollination and fertilization that results in the production of seeds. Seeds are ovules that develop into embryos, store food material, and play a role in plant reproduction. However, seeds have certain issues that need solutions because they face problems like drought susceptibility, dormancy, pest predation, and germination conditioning dependence. Recently in plant biotechnology, artificial seed technology has become popular as a viable method providing answers to several concerns. The main objective of artificial seeds is to provide tissue-grown plants in a greenhouse or field. Micropropagation techniques allow mass propagation and cloning of selected plants which must be acclimated before being placed in a greenhouse or field. Appreciation to artificial

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seeds, somatic embryos from tissue cultures can be placed directly into the soil environment without creating strains that may require an acclimatization period before sowing in the field. This would allow the distribution of elite clonal plant material without incurring the laborious and expensive task of producing transplants. The use of artificial seeds could reduce the cost of tissue culture equipment by providing the opportunity to introduce new plants that can not be propagated using botanical seeds. The impact of such a distribution system on agriculture would be significant as it would combine new technology with greenhouse/field production systems.

In true definition, artificial seed consists of individual somatic embryo covered with a protective coating that mimics the size and shape of real seeds. Earlier, artificial seeds were produced through somatic embryo encapsulation but nowadays these are created by encapsulating different in vitro-derived propagules such as nodal segments carrying axillary buds, apical shoot buds, and stem segments. Though Murashige (1977) was the first to introduce the concept of artificial seeds, he is not credited with being the first to produce dried-up artificial carrot seeds, which was done by Kitto and Janick (1982). Later Redenbaugh *et al.* (1984) made a breakthrough by developing a method of producing synseed by enclosing somatic embryos of alfalfa using sodium alginate. Similarly, Bapat *et al.* (1987, 2005), Mathur *et al.* (1989) and Lal and Ahuja (1995) were able to achieve synthetic seed production from *Morus indica*, *Valeriana walichii* and *Picrorhiza kurroa* shoot buds, respectively, encapsulated in agar and alginate, as substitute of somatic embryos. Synseed technology has gained much attention over the last few decades and successfully applied to numerous plant species, such as forest trees, ornamentals, fruits, vegetables, cereals, and medicinal plants.

BACKGROUND

In higher plants, seeds (or zygotic seeds) are the vehicle that connects one generation to the next. Through seeds, plants can pass on their genetic structure from generation to generation, therefore seeds are the most suitable means of reproduction, protection, and multiplication. Artificial seeds are often described as new equivalents of true seeds, consisting of a somatic embryo surrounded by an artificial shell equivalent at most to an immature zygotic embryo, probably in the post or early cotyledon stage. The possibility for artificial seeds is to be used on a large scale, low-cost agricultural production as an alternative to real seeds. Artificial seeds have many advantages, including superior clonal plants that can be propagated like seeds; it would be possible to preserve rare plant species for biodiversity conservation; and larger, synchronized harvests of important crops would become a reality. Other advantages include ease of handling/processing, the possibility of long-term preservation, and the low cost of production and subsequent reproduction.

The technique of artificial seed production was first used in clonal propagation to grow somatic embryos housed in an artificial endosperm and bounded by an artificial seed coat. Currently, artificial seeds are gel-coated capsules containing not only somatic embryos, apical buds or stem, and root but also axillary segments (Vdovitchenko and Kuzovkina, 2011). Target explants are encapsulated in cryoprotective material such as hydrogel, alginate gel, ethylene glycol, dimethyl sulfoxide (DMSO), and others, which can be processed into a plant. Although the list of advantages of using artificial seeds is long, the commercial potential of the system largely depends on the specific value of the propagated plant and the cost of competing products and technologies. The potential use of artificial seeds will also depend on technological advances in other areas of plant science, such as the development of high-value crops through genetic engineering. However, we can now identify several examples of crops that could greatly benefit from an artificial seed system and focus on markets where true seeds are not available and therefore the high cost of artificial seeds can be supported by market demand.

Need for producing artificial seeds

The primary need associated with such seeds is, on one hand, for many crops, such as fruits, nuts, and certain ornamental plants; it is not possible to produce a true-breeding seed from two parents due to genetic barriers to selfing. Other important criteria relate to the type of artificial seeds that can be produced. Seeds of some tropical crops are recalcitrant (unorthodox) in that they have short viability and must be stored at relatively high moisture content to maintain viability (Sripathy and Groot, 2023). Therefore, for such crops, propagation is accomplished either vegetative by cuttings or the use of relatively low quality open-pollinated seeds.

The production of artificial seeds is essential for several reasons including:

1. Availability of micropropagation protocols for large number of plant species.
2. Artificial seed technology is now recognized as a practical and effective means of propagation in various important crops.
3. Synthetic seeds play a crucial role in directly transferring newly developed plant varieties via biotechnology to fields/greenhouses.
4. Somatic embryos, through micropropagation, can generate a high number of plants, and artificial seeds offer both short and long-term storage capabilities, along with cost-effective transportation methods.

Structure of Synthetic Seed

The main objective of synthetic seed was to address the issue of missing essential accessory tissues like endosperm and protective coatings, needed for storage and handling in somatic embryos. These tissues are crucial for the propagation and preservation of germplasm, making it must for somatic embryos to closely resemble seed embryos. The technology of encapsulation has played a significant role in achieving this goal, with synthetic seeds being composed of two parts - the explant material and the capsule (*Fig. 1*). The explant material can be a somatic embryo, bud, shoot, or any other actively dividing tissue that mimics the zygotic embryo found in true seeds (*Fig. 2*). Differences between natural and artificial seed are given in *Table 1*. Various potentials crops have been used for synseed production given in *Table 2*.

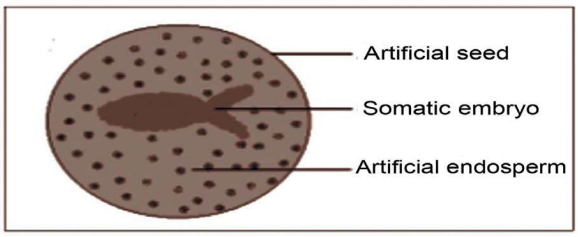

Fig. 1: Artificial Seeds Concept (Redenbaugh *et al.*, 1991).

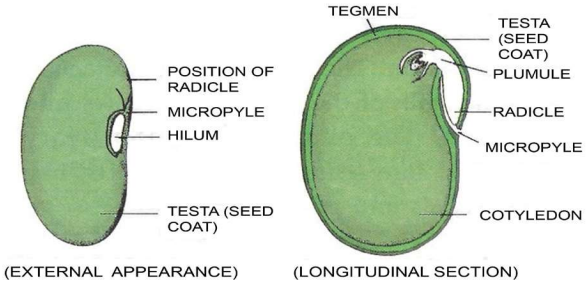

Fig. 2: Structure of a typical natural seed

Table 1: Comparison between artificial seed and natural seed

Natural Seed	Artificial Seed
Presence of hard seed coat	Undergo encapsulation so soft hydrated gel coat
Cotyledons and/or endosperm covers/protect the embryo	No maternal tissue present for the protection of the embryo
Undergo desiccation and dormancy	Do not undergo desiccation and dormancy
Endosperm and cotyledons act as storage reserve and provide nutrition during germination	No tissue is present for storage and encapsulation gels act as source of nutrients during germination

Table 2: Potential crops for artificial seed

Strong Technological basis	Strong Commercial basis (Medium per-unit value)	Strong technological and commercial basis
1. Alfalfa	1. Begonia	1. Celery
2. Caraway	2. Broccoli	2. Coffee
3. Orchardgrass	3. Petunia	3. Corn
4. Panicum	4. Cotton	4. Cotton
Pennisetum	5. Tomato	5. Oil Palm
	6. Geranium	

Essential Requirements for the Production of Artificial Seeds

Creating artificial seeds involves several materials namely Plant Explants, Culture Media, Encapsulation Material, Sterilization Agents, Containers, Growth Chambers, Nutrient Supplements, Tools and Equipment, Storage Containers, and Labels and Markers.

Explant Material(s)

When it comes to creating synthetic seeds, materials like axillary buds, somatic embryos, root and shoot tips, or any other active meristematic tissue can be used. These materials are classified as either bipolar propagules or unipolar propagules given in *table 3*.

Somatic Embryo

Both root and shoot are combined in bipolar structures called somatic embryos. For synseed production, they are ideal due to their polar nature. This allows them to develop shoots and roots simultaneously (Standardi and Piccioni, 1998; Sharma *et al.*, 2013). Because of this single-step capability, somatic embryos make excellent material for synseed production. Somatic embryos

have been successfully used for synseed production in several plant species including *Rotula aquatica*, *Oryza sativa*, Pinus *radiata*, *Nothofagus alpina*, *Dalbergia sissoo*, *Clitoria ternatea*, *Rhinacanthus nasutus, Hemidesmus indicus*, *Anethum graveolens, Mondia whitei, Ledebouria revoluta* and *Curcuma amada*. However, the deficient and asynchronous maturation of the embryonic pole is the basic problem for synseed production in woody species. To address this problem, researchers recommend using compounds such as nutrients, growth regulators, herbicides, antipathogens, biofertilizers, and biocontrollers.

Table 3: Types of propagules

Unipolar Propagules	Bipolar Propagules
Unipolar propagules are specialized plant parts with root or shoot tips that are commonly used to create synthetic seeds. Examples include shoot and root tips, nodal segments, apical or axillary buds, micro shoots, micro bulbs, and rhizomes.	Somatic embryos, one of the most commonly utilized micro propagules, are structured in a way that allows them to develop both root and shoot in a single step. These embryos have a bipolar structure, giving rise to both radical and plumule axes. When a plant is grown from a somatic embryo, it is often called an 'embling'. Furthermore, plant lines originating from somatic embryos maintain their regenerative capacity over an extended period, ensuring consistent plant production.
Advantages 1. Best for synthetic seed production, it can be effectively used in a hydrated and dry state. 2. High regenerative capacity and uniform plant production.	**Advantages of bipolar propagules-** 1. Somatic fragments are easy to obtain 2. Somaclonal variation is reduced
Limitation 1. Asynchronous Growth 2. Embryonic ability is found only in a few genotypes	**Limitations** 1. Spontaneous formation of root 2. Expensive as an additional system for micropropagation is needed

Nodal Segment

Nodal segments with axillary buds, also known as microcuttings, are frequently used for synseed production. This is likely because they are easily produced once the micropropagation system is in place and they can maintain viability for sprouting and conversion even after storage, which is necessary for exchanging germplasm (Piccioni and Standardi 1995; Ahmad *et al.* 2012).

Protocorm-like bodies

These are mainly used in the case of orchids. The protocorms are encapsulated in sodium alginate gel to form synthetic seeds. Different types of explants are used depending upon the species which is listed in *table 4*.

Table 4: List of plant and explants used in synthetic seed

Plant Species	**Explant used**	**Uses**
Carica papaya L.	Somatic embryo	Fruit and medicinal use
Arnebia euchroma	Somatic embryo	Medicinal use
Morus indica L.	Somatic embryo, axillary bud	Industrial use
Zea mays L.	Somatic embryo	Cereals
Cucumber	Somatic embryos	Vegetable crop
Dalbergia sissoo	Somatic embryos	Medicinal use
Grapes	Somatic embryo	Fruit crop
Mondia whitei	Somatic embryo	Medicinal use
Litchi chinensis	Somatic embryo	Fruit crop
Moringa oleifera	Somatic embryo	Medicinal use

table continue

Plant Species	**Explant used**	**Uses**
Camellia sinensis	Somatic embryo	Production of biodiesel fuel
Woody plant species	Somatic embryo	Produce seed
Coriandrum sativum L	Somatic embryo	Spices crop
Pineapple ananas comosus L.	Micro shoot	Fruit crop
Musa paradisiaca L.	Micro shoot	Fruit crop
Rauvolfia tetraphylla	Micro shoot	Medicinal use
Pyrus communis L	Shoot tip	Fruit crop
Helianthus annuus L.	Shoot tip	Industrial/ornamental crop
Picrorhiza kurroa	Shoot tip	Medicinal crop
Ansellia africana	Nodal segment	Medicinal crop
Sphagneticola calendulacea L	Nodal segment	Medicinal crop
Cymbidium finlaysonianum	Protocorms	Fruit
Oryza sativa L.	Androgenic proembryo	Cereals

Matrix

The success of synthetic seeds depends heavily on the composition of the matrix that surrounds the plant material. This matrix provides protection against physical damage and ensures a continuous supply of nutrients, growth factors, and other biological agents needed to safeguard the explants during storage and transportation. When creating synthetic seeds, it is important to use a gelling agent that is not highly toxic, has moderate viscosity, has low spin ability, is cost-effective, and is biocompatible. Alginate is a better choice than agar for long-term storage. Alginate helps in forming capsules and the strong alginate beads provide excellent protection for plant propagules against microbial invasion, mechanical damage, and environmental factors. Several gelling agents like agar, sodium alginate, gelatin, Polycom 2133, carboxymethyl cellulose, and others as given in *Table 5*.

When plant propagules are mixed with sodium alginate and placed in a calcium chloride solution, they form strong and consistent explant beads. The density of the beads is influenced by the amounts and length of time the gelling agents (sodium alginate and $CaCl_2.2H_2O$) are mixed together. These extra ingredients serve as a reserve and gradually merge with the outer layer of the capsule, promoting the growth and development of seedlings. Activated charcoal can also be incorporated into the matrix to enhance the vitality of the enclosed somatic embryos, aiding in the breakdown of the alginate capsule and increasing the respiration of the embryos, which typically lose their strength quickly during the storage period. *Table 5* lists the main components used for synseed.

Table 5: Components used in synthetic seed

Gelling Agents	Complexing Agent	Growth Regulator	Protective Chemical and Microorganism	Coating Agents
Sodium Alginate	Calcium chloride	Gibberellic acid	Rifampicin, Cefotaxime, Tetracycline-HCl	Polylysine
Carrageenan	Potassium chloride	Abscisic acid	Mycorrhiza	Elvax 4260
Locust Bean gum	Ammonium chloride	Zeatin, kinetin	Activated charcoal	Gantrez ES
Gelrite		meta-topolin riboside		Glutaraldehyde
Agar		6-benzylaminopurine		Maleic anhydride
Carboxymethylcellulose		2,4 – Dichlorophenoxy acetic acid		

table continue

Gelling Agents	Complexing Agent	Growth Regulator	Protective Chemical and Microorganism	Coating Agents
Sodium pectate		Indole -3-acetic acid		
Tragacanth Gum		6-benzyl amino purine		
		α-naphthaleneacetic acid		
		Thidiazuron		

Seed Shell

The protective layer surrounding a typical seed is called the testa. This barrier shields the seed from harmful fungi and bacteria, as well as keeps it safe from drying out and physical harm. Synthetic seeds are created using a precise combination of alginate-gelatin, aluminum monostearate, Elvax 4260, Glutaraldehyde, maleic anhydride, Polylysine, or polyproline. This mixture is designed to preserve the ability to store and deliver important additives such as microorganisms, pesticides, herbicides, nutrients, and growth regulators to the plant tissue. Synthetic seeds are created using a precise combination of alginate-gelatin, aluminum monostearate, Elvax 4260, Glutaraldehyde, maleic anhydride, Polylysine, or polyproline. This mixture is designed to preserve the ability to store and deliver important additives such as microorganisms, pesticides, herbicides, nutrients, and growth regulators to the plant tissue.

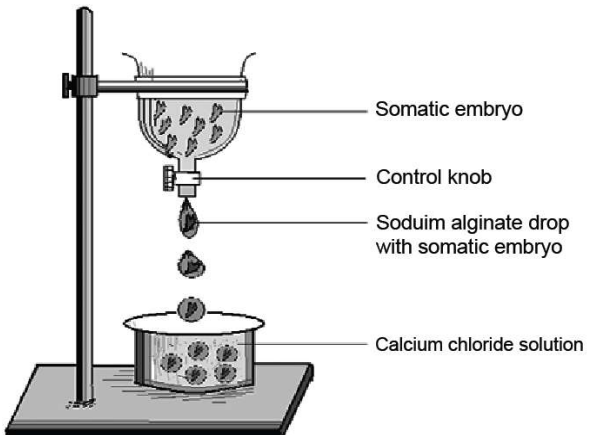

Fig. 3: Dropping Method for artificial seed production

Automatic encapsulation process

An efficient process has been developed to create artificial seeds. Embryos and an alginate solution are mixed and fed into a storage tank. Using a vacuum seeder, these alginate capsules are then sewn into seedling trays *Fig. 4*. The Stanhay planter is a common tool for transplanting these seedlings outdoors. For easy mechanical handling of the seeds, a water-resistant coating is required.

Methods of producing artificial seeds

Dropping Methods

Encapsulation of synthetic seeds via sodium alginate is highly practical. Around 2-3% sodium alginate is dispensed from a funnel into drops. Somatic embryos are inserted into these drops. The embryos are then immersed in calcium salt for approximately 20 minutes. Following this, they are rinsed with sterile water and stored in airtight containers for seed preservation. *Fig 3* shows dropping method for artificial seed production.

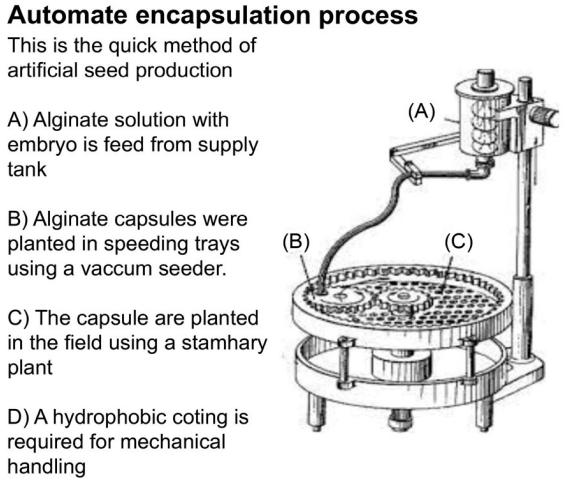

Fig. 4: Automatic Encapsulation Method

Procedure for Artificial Seed Production

Artificial seed production systems could be many depending on the type of artificial seed needed, the demand for such seeds as well as their cost effectiveness among different kinds. Artificial seeds are created using somatic cells, which are non-reproductive cells. A key difference between zygotic and somatic embryogenesis is given in *Fig. 5* and *Table 6*. Steps in Artificial Seed Development

include:

1. Starting the process of creating embryos from somatic cells i.e. somatic embryogenesis
2. Growing mature embryos from somatic cells.
3. Separating and aligning the somatic embryos and large-scale production of embryos.
4. Enclosing mature somatic embryos in a protective shell.
5. Dehydration- Removing moisture from the embryos.
6. Preparing the embryos for planting in the field.
7. Somatic Embryogenesis

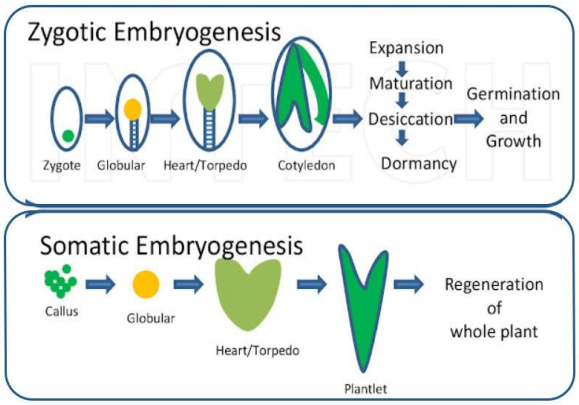

Fig. 5: Zygotic and Somatic Embryogenesis

Table 6: Difference between Zygotic and Somatic Embryogenesis

Somatic Embryogenesis	Steps	Zygotic Embryogenesis
Somatic cell isolated or not Haploid cell (e.g.- microspore)	Origin	Zygote in the ovule
Hormonal Induction Low-level frequency	Initiation	Fecundation (except apomixis) Every zygote Constitutional Polarity Asymmetric Division (Under genetic control)
Similar condition but with variation Absence of suspensor Reorganization in proembryo cluster Adventitious Embryogenesis	Construction of an embryo	Embryo/suspensor Embryo axis in place (Under genetic control)
Interaction between genetic and hormonal control	Meristem Formation	Tightly genetically controlled Root meristem Shoot meristem
Absence of maturation and endosperm External induction Factors are involved	Maturation	Storage protein Dehydration Dormancy Plant maternal tissue interaction

The crops are chosen according to their technical and commercial significance and somatic embryos are made in this case first whereas their maturation is a second step as given in Fig 6. Thereafter mechanization of embryo processing is done. After treating mature embryos to induce dormancy gene expression, encapsulation of embryoids/ embryoids takes place. Depending on whether it is in a greenhouse or in the field, watering, fertilization, transplanting as well as other things may include e.g., greenhouse-to-field transition

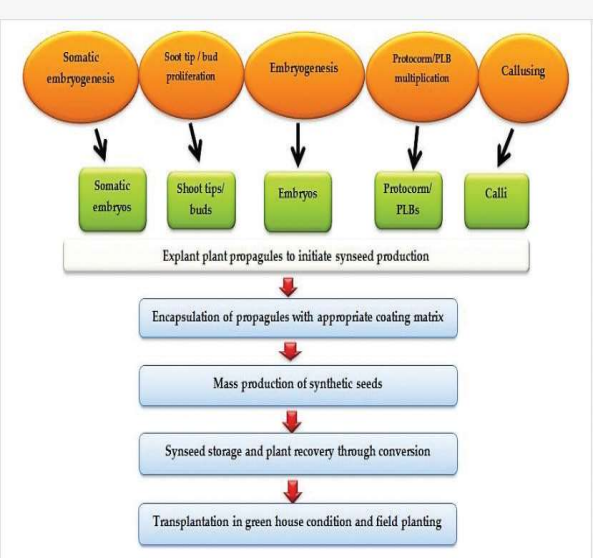

Fig. 6: Procedure for the production of artificial seeds

Process of Somatic Embryogenesis

The somatic embryos have their origin from different parts of plants. By use of plant hormones and special nutrients, these parts of the plant can be encouraged to create disorganized cell masses i.e. callus. The application of suitable hormone(s) enhances the development of somatic embryos from callus. Somatic embryos developed through cell culture are equivalent in terms of development and biochemistry similar to zygotic embryos.

Production of Hydrated Artificial Seeds

In Plant Genetics, Inc., the research on artificial seeds has been restricted to making use of a system that supplies moisture for the embryos

while in a somatic state. Two patents, which included encapsulation of meristematic tissue, like somatic embryos and shoots in gels with water, gels with additional materials, have been issued (Redenbaugh *et al.*, 1986) Several parameters linked to ease of capsule formation and absence of toxicity, like somatic embryos and true seeds, were used to test numerous hydrogels during a certain time period. Sodium alginate, derived from seaweeds, turns out to be the most beneficial gel for somatic embryo encapsulation. Currently, 2% sodium alginate mixed with somatic embryos is employed in producing artificial seeds. Complexing the drops of alginate involves sinking alginate in a solution of calcium nitrate such that the alginate is able to get complexed. It takes about twenty minutes for the somatic embryo to become surrounded by a solid bead formed from the complexed alginate. The completion of the alginate complexation process makes it possible for one to handle little embryos easily, being that the alginate bead acts as a protective barrier for these delicate young plants. A hydrophobic membrane coating can also be developed while encasing the capsule. It aids in the reduction of adhesiveness and enhancing flowability keeping the capsules apart. This is essential as regards machine sowing since the embryo to be covered should lie at certain positions like those for transplant growth in seedling flats.

Planting and Converting Artificial Seeds to Plants

Limited research has been done on the conversion of artificial seeds to plants in a transplant environment. Hence, much of the work has gone into the processes of forming somatic embryos and selecting the gels or matrices that can carry these embryos. Artificial seeds require somatic embryos to be strong enough so that they can survive by themselves when roots, shoots, and leaves are formed with limited evidence since seedling necrosis occurs rapidly even though the overall structure of artificial seeds may seem normal. Embryos should accumulate food reserves for growth or obtain an exogenous nutrient that serves as an artificial endosperm.

The focus is on conducting research and planning the development of prototypes. The main reason for this is disintegrating artificial seeds from these sterile test tubes in which most studies have concentrated so far. It is stressed to achieve a high conversion rate of artificial seeds to plants in a nonsterile soil environment. In this new perspective, the initial step was to identify possible planting techniques as well as cultural practices that would make it possible for artificial seeds to survive in real garden soil. Despite the fact that soil types and watering procedures used in conventional germination of real seeds could also suit imitation seeds, it is important to develop some methods for the specific case.

Important hurdles

Significant obstacle in advancing the commercialization of synthetic seeds is the absence of good somatic embryogenesis systems. These are mostly lacking for high-value genotypes. As advances continue to be made in the realm of reducing the cost of somatic embryogenesis, it stands out that artificial seeds may find it hard to compete economically with inexpensive true seeds hence may, thus, be used only on high-value crops. Therefore, automation of the tissue culture process for embryo production should help decrease the labor-intensive production steps cost.

Encapsulation Method

Encapsulation is important for preventing seeds for drying out. Methods for polymerization and encapsulation to form artificial seeds are given in *fig. 7* and *table 7*.

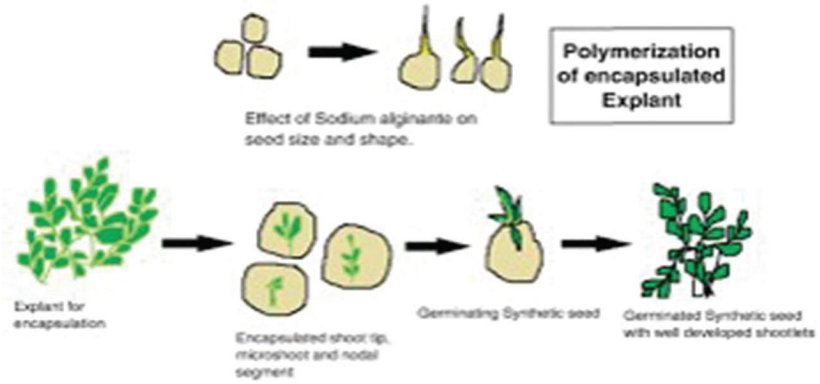

Fig. 7: Polymerization of synthetic seed production

Table 7: Encapsulation method for producing synthetic seeds

Methods	Capsule formed
Complex Coacervation	Yes
Gelation	Yes
Ion Exchange	Yes
Temperature	Yes
Interfacial Polymerization	Yes

Types of Seeds

Desiccated Synthetic Seed	Hydrated Synthetic Seed
The dried artificial seeds were initially developed using somatic embryos, either alone or enclosed in polyox, and then dried out (Kitto and Janick, 1982, 1985). The drying process was done gradually over one or two weeks by placing them in chambers with decreasing humidity levels, or quickly by leaving them exposed overnight on the bench in a laminar airflow chamber (Ara *et al.*, 2000).	The innovative technology of hydrated synthetic seeds was first developed by enclosing hydrated somatic embryos of Medicago sativa in 1984. These special seeds are utilized to cultivate plant species whose somatic embryos are resistant to drying out. Typically, hydrated artificial seeds are made by encapsulating the somatic embryos or other propagules in hydrogel capsules. Various techniques have been explored for creating hydrated artificial seeds, with calcium alginate encapsulation being the preferred method according to Redenbaugh *et al.*, 1993.

Artificial Seed in Bioreactors

Here's a general process of how artificial seeds can be grown in bioreactors:

- Preparation of Artificial Seeds: Artificial seeds are prepared by encapsulating somatic embryos or other vegetative parts such as shoot buds, cell aggregates and axillary buds
- Micro propagules. Under in vitro or in vivo settings, these encapsulated propagules can be sown as seeds and grown into plants.
- Inoculation in Bioreactor: A pre-culture is inoculated into the bioreactor medium. For example, the cells are cultivated atop microscopic hydrogel microspheres. Additionally, they are able to communicate with one another and grow in tiny 3D aggregates.
- Growth and Maturation: The artificial seeds grow and mature in the bioreactor, eventually developing into plantlets.
- Transfer to Soil: Once the plantlets have grown sufficiently, they can be transferred to soil for further growth and development. Researchers efforts on various plants is listed in *Table 8*.

Plant material exchanges between national and international laboratories, as well as the successful preservation of elite plant species' germplasm, have been made possible by this technology along with bulk producing of genotypes/elite plants.

Table 8. Research efforts on artificial seeds

Researcher	Crop	Concept
Kitto and Janick (1982, 1985)	carrot	desiccated, coated embryo clumps
Redenbaugh *et al.* (1986)	Alfalfa, celery	hydrated, encapsulated embryo
Schultheis *et al.* (1986)	sweet potato	hydrated embryos in fluid drilling gels
Gray (1991)	Orchardgrass, grape	desiccated, uncoated embryo

Storage Ability of Artificial Seeds

Seeds have the unique ability to go dormant and store for extended periods of time. Many tropical and subtropical plants with desiccation-sensitive seeds, high cellular metabolism, and susceptibility to pathogens can only be stored for a short time. Using artificial seeds offers a secure way to exchange and conserve plant species. Encapsulation technology is an important strategy in the preservation of explants where explants are maintained in a non-pathogenic environment and for short to long duration. Many means are applied for the purpose of maintaining slow and steady growth in cultures meant for short term storage. One of the ways to ensure that seeds last for a short period of time includes reducing oxygen levels, lowering temperature, reducing light intensity, abscisic acid, the minimization of growth medium, mannitol, sorbitol, and the combination of any of them. Cryopreservation (freezing in liquid nitrogen at -196 °C) stops metabolic activity and serves as a long-term storage method for artificial seeds. Synthetic seeds can be preserved by cryopreservation technique in two ways:

1. **Encapsulation-Dehydration-Technique:** Encapsulation and growth of explants in liquid medium supplemented with sucrose for a period of 1-7 days, after which they are partially desiccated through use of silica gel or drying in the air before freezing quickly in the liquid nitrogen. This remains useful for crops like sugarcane and citrus fruits as well

as Strawberries.

2. **Encapsulation-Vitrification:** Vitrification involves cooling a concentrated solution of cryoprotectant to a cryogenic temperature and then solidifying it on a metastable glass. Vitrification helps protect tissues against damage caused by intracellular ice formation. The encapsulation-vitrification process combines encapsulation, encapsulation-dehydration, and vitrification. Explants placed in alginate beads are impregnated with glycerol and sucrose

Scope of Artificial Seed

Humans are fascinated by artificial seed production owing to their application in agriculture, horticulture, and conservation.

1. **Crop improvement:** Artificial seed production can be used in crop improvement and plant breeding in order to allow for the conservation and propagation of elite plant genotypes that can not be propagated by using common propagation methods like vegetative propagation. This in a way enhances breeding in plants since it enables quicker bursts in number.
2. **Crop Protection:** Artificial seeds can be engineered to encapsulate pesticides, fungicides, or other agents for crop protection. These "bio encapsulated" seeds release the chemicals at the appropriate time, protecting the growing plant from pests and diseases.
3. **Germplasm Conservation:** Artificial seeds provide a means to store, transport and conserve plant genetic material, particularly for rare or endangered plant species.
4. **Hybrid Seed Production:** In hybrid seed production, artificial seeds can be used to efficiently produce large quantities of hybrid seeds. This is especially useful for crops where hybrid vigor is desired, such as corn, rice, and certain vegetables.
5. **Clonal Propagation:** Artificial seeds can facilitate the clonal propagation of plants, allowing for the mass production of genetically identical individuals. This is advantageous for commercial fruit orchards or ornamental crops where uniformity is important.
6. **Micropropagation:** Tissue culture techniques can be combined with artificial seed technology to produce large numbers of plants from small amounts of plant tissue and used for the rapid multiplication of ornamental plants, fruit trees, and other high-value crops.
7. **Bioremediation and Phytoremediation:** Artificial seeds tailored to carry traits that enhance their ability to absorb or break down pollutants, can be used in environmental applications, such as the reclamation of contaminated lands through the introduction of plants with specific remediation abilities.
8. **Nanotechnology Applications:** Advancements in nanotechnology offer opportunities to incorporate enhance nanoparticles into seed coatings to improve germination, nutrient uptake, and stress tolerance.
9. **Regeneration of Plantlets:** Artificial seeds provide a controlled environment for the regeneration of plantlets from somatic embryos or other tissue cultures. This enables the production of plants with desired traits in a highly controlled manner.
10. **Commercialization and Industry Growth:** With the increasing demand for high-quality seeds and the need for sustainable agricultural practices, the market for artificial seeds is expected to grow. This presents opportunities for research, innovation, and commercialization in the field. The future of agriculture and environmental sustainability is promising if research and development in this area is not stopped.

Advantages of Artificial Seeds

1. Use of artificial seeds shows high potential for the multiplication of certain plant species such as orchids and some medicinal species which are incapable of producing viable seeds.
2. It is possible to consider efficient propagation with synthetic seeds as an effective conservation technique for certain rare plant species that include Stevia, which has low seed germination rate due to small size, infertility and non-viability.
3. One of the significant milestones is the in vitro propagation techniques like somatic embryogenesis or micropropagation for the cases of non-flowering plants, genetically modified plants, and lines having difficulty in growing from seeds.
4. It comes with additional advantages like uniformity of plantlets, undifferentiated mass propagation within a short time and non-seasonal production of identical plantlets. The technology offers conduction of gene

banks of elite material outside its natural habitat besides promoting germplasm exchange between countries because it is easy to transport.

5. Somatic embryogenesis is a milestone in the propagation of somatic hybrids, cytoplasmic unstable genotypes obtained through protoplast fusion, and sterile seeds – the latter being sterile species that regenerate by other means. In biology, the development of plants is controlled by master genes while others, such as those responsible for embryogenesis, are conditionally regulated.
6. Hwang *et al.* (2006) examined the development and maturation of somatic embryogenesis and artificial seeds for the commercial aquaculture of endangered brown algae in Korea. Native species that cannot be propagated vegetatively and only yield extremely little amounts of seed can be propagated using this method. For instance, eucalyptus that can withstand salinity in the soil can proliferate in Australia by cutting and genuine seeds.
7. It provides an opportunity for economically viable hybrid seed production in autogamous species (wheat, barley, oats) through mass production using artificial seed multiplication. Somatic embryogenesis is an appropriate technique to reduce labor input in the propagation and production of ornamental plants
8. Commercial crops such as cotton (*Gossypium hirsutum L.*) and soybean (*Glycine max Merril*.) possess expensive hybrid seeds because they bear cleistogamous flowers and abscission problems which result in self-pollination of their seeds. Consequently, these crops have low seed production levels compared to demand. As a solution to this problem, hand pollination is usually done on a few hybrid seeds to increase their quantity thus raising their production cost. This small volume of hybrid seed could be hugely multiplied through artificial seed technology.
9. Synthetic seeds coated with different additives such as herbicides facilitate their storage and transportation without infestation by pests or diseases. Because they are a mix of species with long life cycles, artificial seeds can give life to all the species of pine trees in less time or money.

DISADVANTAGES

1. One of the obstacles is the high price to pay for quality in mass manufacture the of viable micropropagules. This technology can rarely be utilized if tissue dies off or is altered several times whenever somaclonal variations occur.
2. The other problem includes a low production rate of viable micropapules and anomalous and asynchronous development of somatic embryos. A significant limitation is inefficient germination and poor survival which may be attributed to nutrient and oxygen shortage.
3. The conventional encapsulation is labuor intensive; hence it should be replaced by bulk encapsulation that reduces the labor requirements. These problems must be solved in future research in order to increase the acceptance of synthetic seed technology. Also, the economic aspect of the technology is taken care of so that it can be feasible on small scale too.

Artificial seeds production in various crops

In 1989, the technology of synthetic seeds was introduced to the cereal industry in an effort to boost their vigor and yield. The survivability of encapsulated embryos generated from a five-year-old long-term culture of *Oryza sativa cv*. Basmati 370 was investigated by Suprasanna *et al.* (2002).

Hybrid seeds can be pricey for certain types of vegetables, making the plants quite valuable. Pollination must be manually carried out, which demands significant time and effort. Despite the need for extensive time, space, and effort, some species opt for vegetative reproduction. The advancement of artificial seed technology holds promise for cutting costs significantly by decreasing labour, space, time, and materials needed for these plants.

Potato (*Solanum tuberosum*) is a crucial food crop globally, serving as a primary source of food for masses. Due to the challenges associated with using botanical potato seeds, traditional methods of preserving and exchanging potato germplasm are not feasible. Tissue culture-based biotechnology offers an alternative method of propagation, enabling the production and replication of material in a controlled environment. The use of synthetic seeds for potato propagation has been extensively researched to study how different plant parts

(somatic embryos, nodal segments, shoot tips etc.) are enclosed, and to estimate the amount produced and the coverage of *Solanum tuberosum* plants grown in the field (de Moraisi *et al.*, 2018).

Seed propagation in most commercial fruit crops has not been successful due to heterogeneity, small size, reduced endosperm, and scarce germination rate. In certain species, the seeds are desiccation sensitive and recalcitrant which cannot be stored longer than time.

Banana is propagated using suckers that are always produced every year limiting their usage. This system suits artificial seed production. Shoot tips are encapsulated in vitro using 3% sodium alginate solution are preferred as a method of propagation because it has become popular. Papaya is also propagated using encapsulated embryos in sodium alginate. The survival of encapsulated embryos depends on the length of exposure to calcium chloride as well as the concentration of sodium alginate (Radzuan *et al.*, 2019).

Lately numerous crops now available are without seeds. Hassanein *et al.* (2005) and Sandoval-Yugar *et al.* (2009) did the regeneration of encapsulated shoot tips of *Musa paradisica*. For instance, in banana cv. rasthali (Musa spp. AAB group) plantlet regeneration was through alginate encapsulated somatic embryos (Ganapathi *et al.*, 2001).

Synthetic apple rootstock seeds were prepared through utilization of apical and axillary micro-propagated buds. Researchers attempted to mechanize the production of adventitious shoot tips that are fit for encapsulation as a way of economizing labor.

In ornamental plants and orchids, the use of synthetic seeds is particularly attractive since it allows a substantial reduction in seed endosperm. Somatic embryos of two ornamental species, i.e. *Eustoma grandiflorum* and *Genista monosperma*, were used to create synthetic seeds (Ruffoni *et al.*, 1994). Piccioni and Standardi (1995) produced synthetic seeds using shoot tips of *Betula pendula* and bulbs of *Lilium longiflorum*.

Commercial orchids have become the most preferred explants in synthetic seeds preparation including seeds, protocorms, and protocorms like structures. Recently scientists have shown more interest in protocorm-like bodies (PLBs) since they make commercial orchid propagation through synthetic seed systems possible. Two-coat system for encapsulating *Spathoglottis plicata* seeds and protocorms was developed (Khor *et al.*, 1998). One of the most studied methods to produce artificial seeds in alfalfa is encapsulation in hydrogel. This method has been well examined with respect to production of synthetic seeds in *Medicago sativa* by ABA treatment that induces desiccation tolerance and also somatic embryogenesis under different procedures of somatic embryogenesis in alfalfa. In this regard, a study on induction of desiccation tolerance in *Medicago sativa* somatic embryos using ABA treatment, in addition to development of synthetic seeds. A 60% survival rate and regeneration into plantlets are demonstrated by somatic embryos treated with ABA on moist filter paper or sterile soil. Coating and encapsulation of dried alfalfa embryos remain elusive (Redenbaugh *et al.*, 1991).

Artificial seeds of *Coriandrum sativum* were generated from somatic embryos obtained from hypocotyls. Encapsulated shoot buds for *Zingiber officinale* also turned into plantlets (Sundararaj *et al.*, 2010). The germination capacity for man-made seeds of *Coriandrum sativum,* within the groups of spices and plantation crops was 82%, while their survival rate equaled 83% (Chen *et al.*, 1991).

Encapsulated nodal explants of *Camellia japonica* (Janeiro *et al.*, 1997) and shoot tips of *Citrus reticulata* (Antonietta *et al.*, 1998) held in cold storage (4°C) showed shoot proliferation indicating scope for cold storage of such seeds. Researchers have reported somatic embryogenesis induction, synthetic seed production and 70% germination rate in *Elaeis guineensis* (Mariani *et al.*, 2014).

Most of the precious medicinal plants occur in rare and endangered categories. This is because of low fruit and seed set and poor seed germination abilities. Other reasons for rare and endangered plants are urbanization, climate change, habitat modification and pollution. Therefore, conserving these plant species through rapid multiplication is crucial. Propagation via encapsulation of synthetic seeds that contain somatic embryos and vegetative propagules has been described to increase efficiency in producing sufficient plants for replenishment.

Future Perspective

The process of creating synthetic seeds has been a great breakthrough for preserving and multiplying rare, endangered, and vulnerable plant species and allows for long-term storage and efficient multiplication of seedlings, making it extremely valuable in modern agriculture. It has the potential to bring back plant species with elite genetic material that holds significant economic and medicinal benefits for future generations. Additionally, species that do not produce seeds,

such as seedless varieties, can also be propagated using synthetic seed technology. The current state of artificial seed technology in agriculture is still in need of more practical applications to truly push innovation forward. Despite efforts made in recent decades, the focus on applying this technology to preserve elite germplasm and reintroduce it to its natural environment has not been fully successful. The direct planting of synthetic seeds in soil or other commercial substrates like compost and vermiculite is seen as a major hurdle in making this technology more widely applicable. The protective coating of the artificial seed acts as a barrier against drought and diseases, enhancing the longevity of micropropagated plants. It allows for the creation of polyploidy without the need for genetic recombination, making it a valuable tool in plant breeding. When it comes to transgenic plants, creating artificial seeds with somatic embryos can help transfer a single gene from a somatic cell to offspring with the same trait. Research indicates that more studies are necessary to improve non-embryogenic synthetic seeds and refine cultivation techniques for their adaptation to challenging environments. The creation of synthetic seeds offers a promising solution for preserving valuable plant materials over extended periods and preventing the extinction of endangered, rare, and vulnerable plant species (Nandini and Giridhar, 2019).

This artificial seed technology promises the direct use of artificial seeds in in vivo conditions and germplasm conservation. Cloning elite genotypes, such as genetically modified kinds incapable of producing real seeds, is one of the many applications of artificial seed that can boost crop value. Although the process of producing these seeds is quite sophisticated, the plant species is always the determining factor in the initial stage. Additional research is needed to increase root development in non-embryogenic artificial seeds and their cryopreservation capabilities. More research is needed on the development of artificial seeds for cultivation on commercial substrates and in non-sterilized environments.

CONCLUSION

A number of issues still need to be resolved, before this technology can be successfully marketed. One of the major obstacles is the need for mass production of high-quality tiny plant embryos, which is currently a big limitation. Other factors that hinder the successful growth of synthetic seeds include insufficient oxygen and nutrients, invasion by microbes, and damage to plant embryos during production. The methods used have been fine-tuned to produce the desired plantlets effectively. This innovative approach presents numerous benefits, such as cost-effectiveness, lower plantlet expenses, a simple yet scalable technique, the potential for direct application of artificial seedlings in natural environments, and ample storage capacity. The success of this process largely hinges on the plant species involved in the initial stages. Additional studies are needed to improve the ability to culture artificial seeds in commercial substrates and non-sterile conditions to make it commercially viable for micropropagation and germplasm preservation.

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