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Original Article

Tiny Capsules, Giant Impact:Synthetic Seed Technology in Plant Propagation

[1]Bappa Chowdhury* and [2]Dolon Ghosh**

[1]M.Sc. Scholar, Department of Pomology and Post-Harvest Technology,Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar – 736165, West Bengal, India.

[2]M.Sc. Scholar, Department of horticulture, College of agriculture, Govind Ballabh Pant University of Agriculture and Technology, (Pantnagar) Udham Singh Nagar – 263145, Uttarakhand, India.

**Corresponding author: ubkvbappa@gmail.com*

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ABSTRACT

Synthetic seed technology offers a transformative approach to plant propagation by encapsulating somatic embryos, shoot buds, or other meristematic explants within a protective hydrogel matrix most commonly sodium alginate that functions as an artificial endosperm. This article critically examines the theoretical basis, production protocol, agricultural and horticultural applications, documented limitations, and future directions of synthetic seed research. Particular attention is given to species-specific success rates in crops such as sugarcane, banana, orchids, *Citrus*, mulberry, and selected fruit species. Key challenges—including low embryo-to-plantlet conversion rates, desiccation sensitivity, and storage constraints are assessed alongside recent advances in bioreactor-based mass production, cryopreservation integration, and matrix biofortification. This article concludes that while synthetic seed technology holds substantial commercial potential, its widespread adoption requires systematic resolution of protocol inconsistencies, species-specific optimisation, and supportive regulatory infrastructure.

Keywords: *Synthetic seeds • somatic embryogenesis • encapsulation • sodium alginate • germplasm conservation • bioherbicide • plant biotechnology*

INTRODUCTION

Synthetic seeds (artificial seeds) are artificially encapsulated somatic embryos, shoot buds, or other meristematic tissues in a gel matrix (usually sodium alginate), which can germinate and develop into a complete plant. This technology is particularly useful for the propagation of plants that either do not produce viable seeds or have poor germination rates. It also plays an important role in the conservation of elite genotypes and endangered plant species. Moreover, synthetic seeds can be engineered to include nutrients, growth regulators, and even beneficial microorganisms to enhance germination and establishment under field conditions (Singh *et al.*, 2006). Synthetic seeds have wide applications in agriculture, horticulture, and forestry, especially for the propagation of elite genotypes, endangered species conservation, and the exchange of germplasm without the risks associated with pathogen transmission. Despite these advantages, challenges such as low conversion rates, desiccation sensitivity, and storage limitations still need to be addressed for widespread commercial adoption (Ara *et al.*, 2000). Murashige (1977) first proposed the concept of artificial seeds from somatic embryos.

THEORETICAL BASIS AND DEFINITION

A synthetic seed is defined as an artificially encapsulated somatic embryo, shoot meristem, axillary bud, or other regenerable explant that can be stored or sown like a natural seed and can subsequently develop into a complete plant. The term subsumes several synonymous designations found in the literature, including 'artificial seed', 'encapsulated embryo', and 'somaclonal propagule'. Two structurally distinct forms exist: hydrated (encapsulated) synthetic seeds, in which the propagule is maintained within a moisture-rich gel matrix; and desiccated (non-hydrated) synthetic seeds, in which the encapsulated tissue is dried to a low moisture content analogous to orthodox seeds.

The biological basis of the technology rests on the totipotency of plant cells—the capacity of individual somatic cells to regenerate an entire organism under appropriate culture conditions. This property, combined with somatic embryogenesis (the development of embryo-like structures from non-zygotic cells), provides the regenerable propagule material required for synthetic seed production.

TYPES OF SYNTHETIC SEEDS

These are mainly two types.

1. **Desiccated (Non-Hydrated) Synthetic Seeds:** Desiccated synthetic seeds are artificially produced seeds where the encapsulated plant tissue (usually somatic embryos) has been intentionally dried to a specific, low moisture content. They are created to closely mimic the natural state of "orthodox" seeds, which naturally dry out before entering a dormant state. Instead of being kept in a wet hydrogel, the embryos are often coated with a different type of mixture (such as polyoxyethylene glycol) and then subjected to controlled drying. The water content is drastically reduced, putting the embryo into a state of suspended animation (quiescence). (Rai *et al.*, 2022; Verma *et al.*, 2023).
2. **Hydrated (Encapsulated) Synthetic Seeds**: Hydrated (encapsulated synthetic seed) is an artificially manufactured seed consisting of a living plant micro-propagule that is enclosed in a protective, water-rich gel matrix. This matrix acts as an "artificial endosperm," providing the tissue with the nutrients and protection it needs to develop into a complete plant. The plant tissue is enclosed in a hydrogel capsule. The most standard method uses **sodium alginate** dropped into a **calcium chloride** solution, which causes the alginate to instantly cross-link and form a firm, spherical gel bead around the embryo. The hydrogel matrix keeps the plant tissue wet and metabolically active.

COMPONENTS OF SYNTHETIC SEEDS

I. Propagules:

(Also Known as Explant) They are different types:

- **Somatic embryo (most common):** Somatic embryos are the most widely used and ideal propagules in synthetic seed technology due to their high regeneration potential. They are derived either from somatic embryogenesis of callus tissue or through direct embryogenesis from explants without an intermediate callus phase. These embryos are structurally bipolar, possessing both shoot and root meristems, which enables them to develop into complete plants without the need for additional organogenesis (Redenbaugh *et al.*, 1991).
- **Shoot apical meristem (SAM):** The SAM consists of actively dividing meristematic cells located at the shoot tip and retains a high regenerative capacity, enabling the direct development of shoots under suitable culture conditions. Due to its inherent ability to maintain genetic stability and continuous growth, SAM is considered a reliable alternative to somatic embryos for artificial seed production (Ara *et al.*, 2000)**.** This approach has been successfully applied in crops such as banana, sugarcane, and certain *Citrus* species, where somatic embryogenesis is either inefficient or difficult to achieve (Reddy *et al.*, 2012).
- **Axillary buds:** These buds arise from the axils of leaves and possess pre-existing meristematic tissue, which allows direct shoot regeneration without passing through a callus phase. This characteristic significantly reduces the chances of somaclonal variation, making them a preferred choice over somatic embryos in certain species (Redenbaugh *et al.*, 1991). Axillary buds are widely utilized in crops such as grape and potato in synthetic seed protocols, where genetic uniformity and true-to-type plant production are essential for maintaining varietal characteristics (Reddy *et al.*, 2012).
- **Protocorm-like bodies (PLBs):** These are specialized propagules used in synthetic seed technology, primarily for orchid species due to their unique embryogenic tissue characteristics. PLBs are analogous to somatic embryos in orchids and originate from meristematic tissues during in vitro culture. They possess high regenerative capacity and can readily develop into complete plantlets under suitable conditions (Ara *et al.*, 2000). The use of PLBs is largely restricted to orchids because of their distinct developmental pathway, which differs from typical somatic embryogenesis observed in other plant species (Reddy *et al.*, 2012).
- **Micro-shoots and nodal segments:** are used as alternative propagules in synthetic seed technology, particularly when somatic embryogenesis protocols are not well established for the target species. These explants contain pre-formed meristems, which enable direct shoot regeneration without the need for callus formation, thereby ensuring faster and more reliable plant development (Redenbaugh *et al.*, 1991).

II. Encapsulation Matrix:

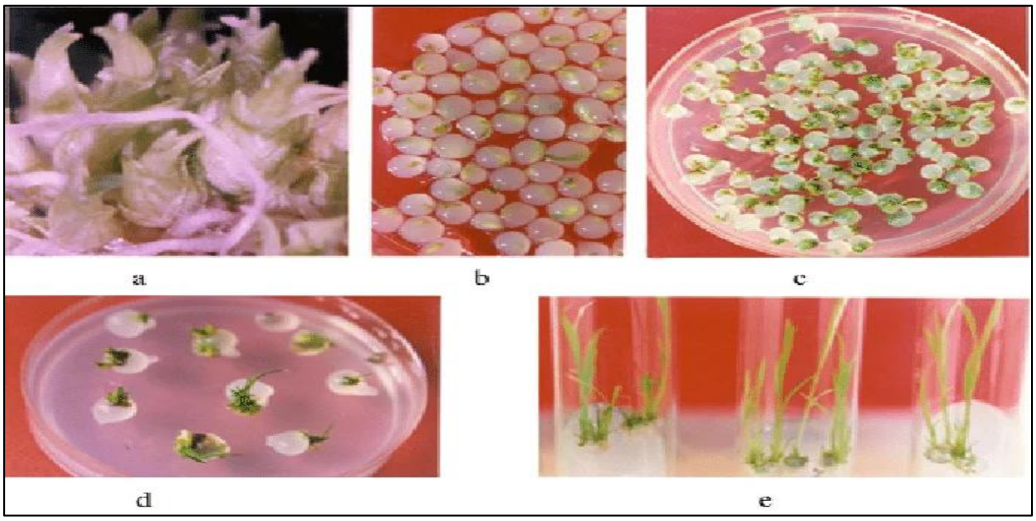
The encapsulation matrix is the artificial coating or hydrogel that surrounds somatic embryos or other propagules such as shoot tips and buds, converting them into synthetic seeds. This matrix provides mechanical protection, maintains moisture, and creates a suitable microenvironment that supports growth and regeneration of the encapsulated propagule (Chaudhuri, 2020; Qahtan *et al.*, 2019). The most commonly used encapsulation system involves sodium alginate (2–4%) combined with calcium chloride ($CaCl_2$ ~0.1 M), where ion exchange leads to the formation of firm, bead-like structures suitable for handling and germination. Other gelling agents such as carrageenan, polyacrylamide, gelrite, and agar have also been used depending on the species and protocol requirements (Chavan *et al.*, 2021; Karthik, 2023).

III. Artificial Endosperm:

The artificial endosperm refers to the nutrient-enriched medium incorporated within the encapsulation matrix of synthetic seeds, designed to mimic the function of natural endosperm in supporting embryo development. This internal nutrient supply provides essential elements required for the growth and germination of the encapsulated propagule, especially under in vitro or ex vitro conditions (Karthik *et al.*, 2021; Singh *et al.*, 2022). the artificial endosperm consists of Murashige and Skoog medium (MS medium) supplemented with sucrose as a carbon source, along with plant growth regulators such as BAP (6-benzylaminopurine) and IBA (indole-3-butyric acid). In addition, vitamins and amino acids are included to enhance metabolic activity and improve the conversion of synthetic seeds into viable plantlets (Chaudhuri, 2020; Karthik, 2023).

IV. Protective Coating:

Protective coating refers to an additional outer layer applied over the encapsulated alginate beads of synthetic seeds to enhance their durability, desiccation tolerance, and storage life. This coating acts as a barrier against moisture loss, mechanical damage, and microbial contamination, thereby allowing the synthetic seeds to be stored and handled under non-hydrated (desiccated) conditions (Singh *et al.*, 2021). Materials such as polyoxyethylene stearate, Elvax 4260 (ethylene-vinyl acetate copolymer), and wax coatings are commonly used for this purpose. These substances help regulate water loss and maintain the viability of the encapsulated propagules during storage and transport. The application of such coatings is particularly important for developing desiccation-tolerant synthetic seeds, which can be stored for extended periods without immediate germination requirements (Rai *et al.*, 2022; Verma *et al.*, 2023).



Pictorial depictions of synthetic seed production and plantlet regeneration in rice (Roy, 2002)

Note: (a) Stereomicroscopic view of germinating micro tillers; (b) Encapsulated micro tillers in sodium alginate (4%) beads; (c) Mass germination of beaded micro tillers on MS medium with no hormones; (d) Seedling elongation from germinating synthetic seeds; (e) growing plantlets under in vitro culture condition on MS basal medium.

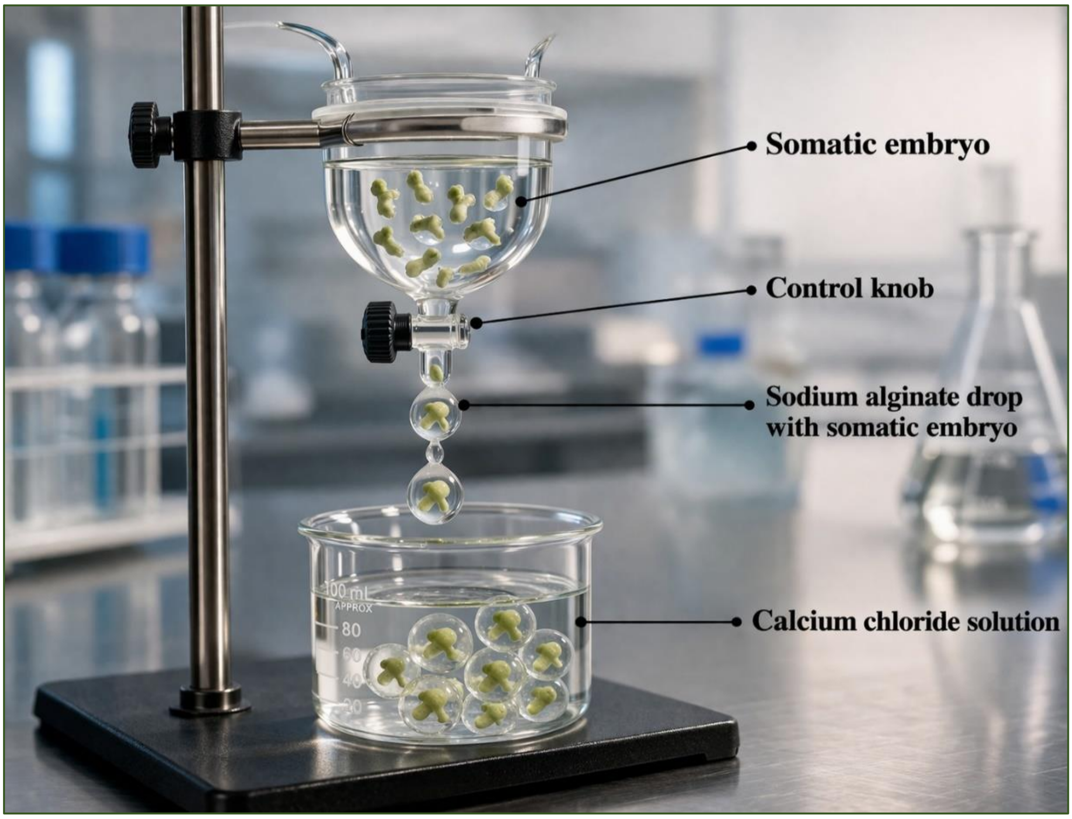


Fig: Encapsulation Technique

ENCAPSULATION TECHNIQUE

Encapsulation is the critical step in synthetic seed production, involving a chemical gelation process that transforms liquid sodium alginate into a solid protective matrix. When drops of sodium alginate containing the propagule are introduced into a calcium chloride ($CaCl_2$) solution, calcium ions (Ca^{2+}) rapidly replace sodium ions (Na^+), resulting in ionic cross-linking of the alginate polymer chains. This reaction forms a firm, water-insoluble, spherical gel capsule around the embryo or other plant tissue (Karthik *et al.*, 2023; Singh *et al.*, 2022). This process, commonly referred to as ionic gelation, produces uniform beads that encapsulate the propagule and provide mechanical protection along with a controlled microenvironment. After formation, the encapsulated beads are allowed to remain or "cure" in the calcium chloride solution for approximately 10–30 minutes to achieve the desired hardness and structural stability (Rai *et al.*, 2022; Verma *et al.*, 2023).

Steps Of Synthetic Seed Production

Step	Stage	Procedure and Critical Parameters
1	Explant Selection	Select disease-free elite mother plants. Excise nodal segments, leaf explants, or zygotic embryos. Surface-sterilise with 0.1% $HgCl_2$ (2–3 min) or 70% ethanol (30 s); rinse 3× with sterile distilled water.
2	Callus Induction	Culture explants on MS medium + 2,4-D (0.5–2.0 mg L^{-1}) at 25 ± 2°C, 16 h photoperiod. Embryogenic callus forms in 3–6 weeks.
3	Somatic Embryogenesis	Transfer callus to auxin-free MS + BAP (0.5 mg L^{-1}). Development progresses: globular → heart → torpedo → cotyledonary stage. Cotyledonary-stage embryos are selected for encapsulation.
4	Synchronisation	Sieve or manually select embryos at a uniform developmental stage. Density-gradient centrifugation or size-selective sieves produce a homogeneous population, improving downstream conversion rates.
5	Mass Production	Scale up via suspension culture (liquid MS) or bioreactor (5–10 L). Agitate at 100–120 rpm; embryo density 500–1,000 per ml.
6	Matrix Preparation	Dissolve 3% sodium alginate in MS liquid + sucrose (3%) + BAP (0.5 mg L^{-1}) + IBA (0.1 mg L^{-1}) + vitamins. Autoclave; cool to room temperature before use.
7	Bead Formation	Drop embryo–alginate suspension (sterile pipette or automated dropper) into 100 mM $CaCl_2$ solution. Ionic gelation produces firm spherical beads (~4–5 mm diameter).
8	Curing and washing	Cure beads in $CaCl_2$ for 20–30 min. Wash 3× with sterile distilled water to remove excess calcium. Transfer to sterile Petri plates.

9	Quality Assessment	Evaluate bead uniformity, surface smoothness, embryo viability (TTC test), germination frequency (target: ≥80%), and conversion rate on MS medium within 2 weeks.
10	Storage and Deployment	Hydrated seeds: store at 4°C for up to 60 days. Desiccated seeds: dry to 10–15% moisture content; store at room temperature for months. Germinated plantlets → hardening chamber → field transfer.

Table-1: Steps in synthetic seed production

APPLICATIONS IN AGRICULTURE AND HORTICULTURE

- ***Clonal Propagation of Elite Genotypes:***

Synthetic seed technology enables the large-scale clonal multiplication of genetically superior or high-value plant varieties without the genetic recombination that accompanies seed propagation. This is particularly valuable in fruit crops where elite rootstocks, disease-resistant scions, or high-yielding selections must be preserved identically across generations (Singh *et al.*, 2006).

- ***Germplasm Conservation:***

Synthetic seeds combined with cold storage or cryopreservation represent a viable strategy for ex situ conservation of endangered, rare, or economically important plant species. Medium-term storage at 4°C and long-term storage in liquid nitrogen (−196°C) have both been demonstrated for selected species, offering alternatives to conventional seed banks where conventional seed viability is problematic (Karthik *et al.*, 2023).

- ***Pathogen-Free Propagation and Germplasm Exchange:***

Encapsulation in a synthetic matrix allows plant materials to be moved across regions or national boundaries with reduced phytosanitary risk compared to conventional vegetative material, because the protocol incorporates surface sterilisation and controlled culture conditions. This is particularly relevant for vegetatively propagated crops such as banana, sugarcane, and orchids, which are prone to systemic pathogen accumulation (Reddy *et al.*, 2012).

- ***Crop-Specific Case Evidence***

Crop Species	**Propagule Used**	**Key Outcome**	**Reference**
Sugarcane	Shoot apical meristems	High multiplication rates; maintained clonal fidelity under SAM encapsulation; virus-free plants achieved	Reddy *et al.*, 2012

Crop Species	**Propagule Used**	**Key Outcome**	**Reference**
Banana	SAMs / somatic embryos	Successful ex vitro conversion; reduced production cost vs. conventional micropropagation	Reddy *et al.*, 2012
Orchid (*Dendrobium*)	Protocorm-like bodies	PLBs encapsulated and germinated at >70% conversion; commercially scalable in Taiwan	Ara *et al.*, 2000
Citrus spp.	Somatic embryos / SAMs	Nucellar embryos encapsulated for rootstock production; 60–75% conversion reported for *C. sinensis*	Redenbaugh *et al.*, 1991
Mulberry (*Morus alba)*	Axillary buds	Vigorous shoot regeneration; 4°C storage for 45 days with >80% viability	Singh *et al.*, 2006
Grapevine	Axillary buds	Genetic uniformity maintained; axillary bud encapsulation preferred over somatic embryos	Redenbaugh *et al.*, 1991
Alfalfa *(Medicago sativa)*	Somatic embryos	First field trial of synthetic seeds conducted by Redenbaugh *et al.* (1986); conversion rates of 30–50% under optimal conditions	Redenbaugh *et al.*, 1991
Potato	Axillary buds / micro-shoots	True-to-type Mini tuber replacement; 85% conversion ex vitro under optimal humidity	Reddy *et al.*, 2012

Table 2. Selected crop systems where synthetic seed technology has been applied, with reported outcomes.

CHALLENGES AND CRITICAL LIMITATIONS

- ***Low and Inconsistent Conversion Rates:***

The most frequently cited limitation is the gap between germination frequency (the percentage of synthetic seeds that germinate) and conversion rate (the percentage that successfully develop into transplantable plantlets). While germination frequencies of 70–90% are achievable under carefully controlled in vitro conditions, conversion rates under ex vitro or field conditions are often substantially lower—sometimes below 30–50% even in well-studied systems such as alfalfa

(Redenbaugh *et al.*, 1991). The primary causes include asynchronous embryo development, incomplete embryo maturation, and the physiological stress of transition from in vitro to ambient conditions.

- ***Desiccation Sensitivity and Storage Problems***

Hydrated synthetic seeds must typically be used within 30–60 days of preparation when stored at 4°C. This short shelf life is a major practical constraint for distribution logistics. The development of desiccated synthetic seeds addresses this problem in principle, but achieving desiccation tolerance is highly species-specific. Many species that support excellent somatic embryo production—including many fruit crops—cannot withstand the moisture reduction required for long-term desiccated storage without unacceptable viability loss (Rai *et al.*, 2022). Even moderate drying (to 30–50% moisture content) causes membrane damage, protein denaturation, and free radical accumulation in desiccation-sensitive propagules.

- ***Species-Specific Protocol Requirements***

No universal synthetic seed protocol exists. The optimal concentrations of sodium alginate, gelling agents, growth regulators, carbon sources, and physical parameters (pH, osmolarity, temperature) differ substantially between species and even between genotypes of the same species. Developing a reliable protocol for a new crop species typically requires extensive and time-consuming empirical optimisation. This has prevented synthetic seed technology from being easily transferred from model systems (alfalfa, carrot) to commercially important but recalcitrant species, including many woody perennial fruit crops (Chavan *et al.*, 2021).

- ***Contamination and Microbial Risks***

The nutrient-rich gel matrix of hydrated synthetic seeds provides an ideal substrate for bacterial and fungal contaminants, particularly during ex vitro germination or field sowing. Even under stringent aseptic conditions, contamination rates can exceed 15–20% in some protocols. The inclusion of antimicrobial agents in the matrix—while partially effective—risks suppressing propagule metabolism and reducing conversion rates.

- ***Scale-up and Economic Viability***

The economics of synthetic seed production are favourable only when compared to conventional vegetative propagation methods that are inherently slow or disease-prone. When conventional seed production is viable, synthetic seeds are rarely cost-competitive. The capital requirements for bioreactor-based mass production systems represent a significant entry barrier for small-scale nurseries in developing countries, limiting commercial adoption to large agribusiness operations (Verma *et al.*, 2023).

FUTURE PROSPECTS

The integration of synthetic seed technology with Cryopreservation is considered one of the most promising approaches for indefinite conservation of elite germplasm. Storage in liquid nitrogen (−196°C), combined with cryoprotectants such as DMSO, glycerol, and sucrose, enables long-term preservation of genetically superior clones in gene banks (Karthik *et al.*, 2023; Singh *et al.*, 2022).

Another major advancement is the use of bioreactor-based mass production systems for somatic embryogenesis. Automated systems, including temporary immersion bioreactors, allow large-scale production of uniform propagules while significantly reducing production costs and labour requirements (Rai *et al.*, 2022; Verma *et al.*, 2023).

Biofortification through matrix engineering is another key direction, where essential micronutrients such as iron and zinc, along with bio stimulants like humic acid and seaweed extracts, are incorporated into the artificial endosperm. This enhances early seedling vigour and establishment under field conditions (Rai *et al.*, 2022; Singh *et al.*, 2022).

In the context of climate change, synthetic seed technology can be utilized to propagate stress-tolerant rootstocks, such as drought-tolerant *Citrus* and flood-tolerant mango genotypes, for cultivation in climate-vulnerable regions (Singh *et al.*, 2022; Rai *et al.*, 2022).

Finally, the development of a robust regulatory framework by organizations such as Indian Council of Agricultural Research, National Bureau of Plant Genetic Resources, and Department of Agriculture Cooperation and Farmers Welfare will be essential to ensure quality standards, biosafety, and commercialization guidelines for synthetic seed products (Verma *et al.*, 2023; Singh *et al.*, 2023).

CONCLUSIONS

Synthetic seed technology represents a genuinely significant advance in plant propagation science, with demonstrated utility across a range of agronomically and horticulturally important crop systems. Its core contributions enabling the clonal perpetuation of elite genotypes at scale, facilitating pathogen-free germplasm exchange, and providing ex situ conservation options for endangered species address real and pressing needs in modern agriculture.

However, the gap between laboratory performance and commercial viability remains substantial. The three most critical barriers are: (1) inconsistent and often low embryo-to-plantlet conversion rates under ex vitro conditions; (2) desiccation sensitivity limiting storage life for the majority of crop species; and (3) the absence of universal protocols, necessitating time-intensive, species-specific optimisation for each new crop target. These limitations are not insurmountable, but they require sustained, focused research effort rather than iterative incremental work.

Future research should prioritise: systematic comparison of encapsulation matrix compositions across diverse crop species; development of standardised desiccation tolerance assays; integration of bioreactor production with cryopreservation to create economically viable long-term storage pipelines; and construction of regulatory and certification infrastructure to enable commercial-scale operations. Progress in these areas will be necessary before synthetic seed technology can transition from a research-phase innovation to a routinely deployed tool in horticulture and agricultural biotechnology.

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