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# Exploring the potential of synthetic seeds: Influence of explant, encapsulating agent and matrix, advantages and challenges

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## Abstract

Synthetic seed can serve as a substitute for conventional seed where conventional seed production is not practical. This method gives a viable solution for propagating plants that are difficult to reproduce through traditional means. Technologies based on synthetic seeds, encapsulating somatic embryos, shoot tissues, or axillary buds in a suitable matrix, demonstrate great influence over plant propagation by helping to accelerate germplasm exchange, increasing genetic preservation, and efficient genetic modification, thus providing the avenue for planting new seeds and accomplishing common goals. This review paper explores the importance of synthetic seeds, the impact of different explants, matrix composition, and encapsulating agents on the quality of synthetic seeds, as well as the benefits and drawbacks of synthetic seeds. Among the various explants used in synthetic seed production, somatic embryos promote genetic stability, shoot buds possess better viability, and axillary buds ensure genotype conservation. Alginate is the prevalent encapsulating agent due to its biocompatibility and cheapness. However, variable germination rates and microbial contamination remained a challenge and we must develop a protocol standardization too. Besides techniques like enhancing the germination rates, stabilizing genes, and having secondary metabolites in the process, the use of cryopreservation technologies and field performance evaluation is also crucially important in the process of creating synthetic seeds. This review discusses current trends in synthetic production research, emphasizing the need for new strategies to address poor germination rates and standardize explants used in synthetic seeds. It examines the factors affecting the production of synthetic seeds, factors affecting seed quality, and potential future developments.

## Keywords

synthetic seeds; somatic embryos; shoot tissues; axillary buds; encapsulating agents; explant; matrix

## Introduction

Artificial seeds, also known as synthetic seeds, are synthesized by transplanting somatic embryos, shoot buds or axillary buds into specific media. Synthetic seeds can therefore increase the number of plant species by producing massive quantities, preserving genetic materials and modifying the genetic material of plants. Reviewing the synthetic seed technology and the traditional propagation processes reveals several benefits including fast

germplasm exchange and disease free and healthy plant types. This method makes it simple to improve variables including biodiversity, rapid species conversion, and cultivation time and cost (1).

The idea of synthetic seeds was first offered by Murashige in the year 1974, where he considered the possibility that the encapsulation of somatic embryos might act as a substitute for zygotic embryos (2). After that, synthetic seeds have been made from various types of explants like somatic embryos, shoot buds, axillary buds, cell aggregates etc. However, the successful development of synthetic seeds was reported in the early 1980s in the case of carrot (3) and alfalfa (4). Later, it was expanded to include the use of numerous non-embryogenic propagules, including roots (5), shoot tips (6), nodal segments (7) and microplants/cuttings (8). This is because not all culturable plants have the capacity for somatic embryogenesis. Nevertheless, synthetic seed production is likely to be affected by factors like the type of encapsulating agent, choice of explants and matrix composition. These conditions determine to what degree of viability, germination and conversion of synthetic seeds can take place, and their morphological, physiological and biochemical traits (1, 2).

Medicinal plants contain a wide spectrum of natural products with an array of advantageous properties. Numerous medicinal plants currently suffer from serious threats, such as overharvesting, destruction of their habitat, environmental pollution, and consequences of it. Nevertheless, it is essential to acknowledge that these obstacles are severely affecting the future existence and production of these plants for the coming generations. Synthetic seed technology can provide a reliable and economical answer to the problems associated with conventional conservation and utilization methods, like low seed viability, poor seed germination, slow multiplication rate, seasonal dependence and genetic erosion. The synthetic seeds can also be used for the genetic improvement of plants. They do so by putting together desired traits through somatic hybridization, gene transfer, or mutagenesis (9).

### *Explant selection*

The selection of explants is the inherent factor that controls the effectiveness of synthetic seed production. Explants are plant tissues that are used as a cell source for capsulation. Many types of explants can be used for the manufacture of synthetic seeds, consisting of somatic embryos, shoot buds, axillary buds, cell aggregates, protoplasts etc. Nevertheless, each type of explant has its benefits and drawbacks, which depend on the plant species, encapsulating agent, and composition of a matrix.

Somatic embryos are the most common explants applied to produce the synthetic seeds since they are close to zygotic embryos in terms of their morphology and their development. Somatic embryo formation can occur from various tissues like leaves, stems, roots etc, depending upon the experiment conditions and plant growth regulators. Somatic embryos hold several benefits, including the ability to germinate and grow into plants without having to go through preceding intermediate steps, high genetic stability and uniformity and the convenience of handling and encapsulation. Nevertheless, somatic embryos also encounter some challenges, for example, insufficient viability and germination rates, the lack of tolerance to drought, the powerful impact of microbial, and the development of some abnormalities like fused, malformed or vitrified embryos (10).

Shoot bud propagation is yet another method of *In vitro* production of seeds. The shoot-forming buds or meristematic tissues can further develop into shoots and leaves. It is possible to obtain the shoot buds from various sources including *In vitro* cultures, axillary buds, and adventitious buds. The shoot buds are better than the somatic embryos and the quality of the shoot buds is good in the respects of the higher viability and germination rate, and the better desiccation tolerance. Although these shoot buds have some drawbacks, such as additional procedures required before conversion into seedlings, low genetic integrity and inconsistent performance, and the difficulty of handling and encapsulation owing to their size and shape, they are still a promising regeneration technique (11).

Axillary buds are an unusual type of shoot apexes found at the bases of the leaves or bracts. Axillary buds from plants with a sympodial growth habit, like orchids, bananas, and pineapples, can be used for synthetic seed production. Axillary buds have the same advantages and disadvantages as the shoot buds but, at the same time, have a lot of the extra benefits of the generation of multiple shoots and plants, conservation of the original genotype and phenotype and compatibility with cryopreservation (11).

In addition to tissue explants, cell aggregates can be another type of explants used for artificial seed production. Cell aggregates are comprised of cell clusters stemming from callus and suspension cultures. Cells aggregates allow for plant synthetic seed production, including ginger, turmeric and garlic. A few of the advantages of cell aggregates include high multiplication rate, ease of handling and encapsulation and the possibility of genetic alteration. Although cell aggregates possess some advantages, such as high rates of viability and germination, the loss of desiccation tolerance, and susceptibility to microbial infection, they also cause soma clonal variations (12).

The protoplast is also an alternative explant that can be utilized in the synthesis of artificial seeds. Protoplasts are cells that have been stripped of their cell walls either by enzymatic or mechanical methods. Protoplasts can be used for artificial seed production of those plants with high somatic embryogenesis potential, like carrots, tobacco, citrus etc. Protoplasts have some advantages of fusion with other protoplasts or cells, genetic modification via genetic engineering, and handling and encapsulation are also easy. On the other hand, protoplasts also have serious drawbacks like low viability and germination rates, not being tolerant to desiccation, and being prone to microbial contamination and soma clonal variation (13).

The selection of explants for synthetic seed manufacture relies on the type of the plant, the specific encapsulating agent, and the composition of the matrix. Often, different types of explants require different methods for encapsulation, germination and conversion. Hence, it is important for each type of explant and each plant species to be optimized to obtain better results.

### *Encapsulating agent*

The encapsulating agent is the material that is used to cover the explant and also to establish a suitable environment for the growth and in the creation of explants (14-16). The optimal concentrations for the firm, clear beads are typically 3% sodium alginate and 100 mM calcium chloride (14, 17). The encapsulating agent plays a very crucial role in the process of synthetic seeds, as it is responsible for viability and germination as well as not only the shape, physiology, and biochemistry but also the process that transforms synthetic seeds into authentic seeds (9, 10, 18).

The criteria for selecting a suitable encapsulating agent for synthetic seed production are as follows:

- It should be biocompatible, biodegradable, and nontoxic to the explant and the environment (19).
- It is supposed to display a good gelation property, like setting at room temperature and having uniformity and stability during storage and handling (20).
- The medium of nutrients needs to be in such a state where permeability can allow easy passage of gases, water, and nutrients that are to and fro from the explants and the external environment (19).
- The strength should be good, including the ability to resist the pressure during capsulation, storage, germination etc (19, 21).
- It should have good optical properties, such as transmission of light for the metabolic processes of plant cells and induction of the growth of explant (14).
- This should be of low price and convenient (14).

The most employed encapsulating agent for the synthesis of synthetic seeds is alginate, which is a natural polysaccharide extracted from brown algae. Alginate is preferred due to its ability to enhance capsule formation and provide sufficient firmness to protect propagules from mechanical injury (19). Alginate offers a couple of great properties, which include biocompatibility, biodegradability, non-toxicity, good gelation properties, good permeability, good mechanical strength and good optical characteristics (14). Besides, alginate is inexpensive and easily acquired (14). Alginate gels by forming a cross-link with divalent cations, including calcium, magnesium or barium. The quick and easy gelation of alginate, which doesn't require heating or cooling, is one of its main advantages. The solidity and viscousness of alginate can be regulated by changing the concentration of alginate, the kind and concentration of cations, and the solution's pH (10). Sodium alginate concentrations of 2-3% are typically optimal for creating firm, round capsules that protect against mechanical injuries (21-23).

A variety of other encapsulating materials have been used in synthetic seed formation that include agar, gelatin, carrageenan, pectin, chitosan, cellulose, starch etc. However, these agents have some constraints, such as poor formation property, poor permeability, not being resilient to movement, poor optical properties, high cost, and low availability. In these cases, they also contain some agents that may be activated and gelled by heating/cooling or even acting as an influence on the viability and development of the explants. With this, they are thus combined with alginate to further enhance their performance or are used with additives like activated charcoal, polyethylene glycol, and sucrose to improve their activity (24).

Capsule agent is the most crucial element in the process of synthetic seed making. It should fit the plant species, the type of explants, and the matrix composition. Encapsulants shed can have a variable impact on the functioning and usefulness of artificial seed products. Hence, it must be done to select the conditions that work well for each encapsulant and each plant species such that they can thrive appropriately.

### *Matrix composition*

Synthetic seeds are produced by encapsulating plant micropropagules in a protective matrix, typically calcium alginate gel (19). The matrix composition can be defined as the mixture of the encapsulating agent and other additives that are accommodated within the gel making it around the explants. The matrix composition leads to the changing of the physical, chemical, and biological features of the synthetic seeds, like the gel strength, viscosity, porosity, water content, pH, osmotic potential, nutrient supply etc. Apart from changing the suitability, germination, viability, growth and characteristics of the synthetic seeds, the particular composition also regulates the morphology, physiology, and biochemistry (19, 25). The composition of this matrix significantly affects seed formation, germination, and subsequent plant growth. Key factors include the concentrations of sodium alginate and calcium chloride, exposure time to calcium chloride, and the addition of growth regulators like benzyl adenine (26). The inclusion of plant growth regulators like IBA and GA3, along with appropriate sucrose concentrations, can improve synthetic seed germination and conversion (27).

The matrix composition for synthetic seed production can be optimized by changing the following factors:

### The concentration of the encapsulating agent

The amount of the embedding agent can be changed to make the gel stronger or fluid. The higher the concentration of the encapsulating agent, forms the gel that is thicker and dispersed the gel, hence giving the cutlets a protection guard against mechanical injury and dehydration. Optimal sodium alginate concentrations range from 2.5% to 3%, with lower concentrations producing fragile beads and higher concentrations resulting in harder, tail-forming

beads (9, 28). While a higher amount of encapsulating agent would lower the permeability of the gaseous exchange and nutrient uptake of the explants, on the other hand, it would have lower porosity of the matrix, and therefore lower the permeability. Hence, the right amount of gel strength with the matrix permeability should be done so that this need for each plant species or type of explant is met. Concerning the optimal concentration levels of the encapsulating agent for synthetic seed creation, we should keep in mind that when the value of the concentration level ranges from 1% to 5%, it differs from one type of encapsulating agent to another and from one plant species to other species (13,19). The matrix composition affects germination rates and plantlet development. Adding active charcoal to MS medium improved conversion rates in long pepper synthetic seeds (29). For garlic, MS medium with growth regulators enhanced shoot proliferation from encapsulated bulblets (28). The gel strength, which is based on calcium chloride ($CaCl_2$) and sodium alginate concentrations, influences the response of vegetable sweet potato nodes. Encapsulation using 100 mM $CaCl_2$, and 4% sodium alginate produced firm, rigid beads that were ideal for root and shoot emergence. The emergence of shoots and roots was delayed by tougher, less permeable beads made with higher concentrations of $CaCl_2$ (120 mM) or sodium alginate (5%) (30).

### The type and concentration of cations

The existence and intensity of cations will cause gelation and cross-linking among the encapsulated fractions. Varying in size and charge of cations, such as calcium, magnesium, barium and others, have their way of influencing the gel properties and the development of the explant. $Ca^{+2}$ used in the form of $CaCl_2$ is the most used cation for synthetic seed production because it forms a stable and uniform gel, and it is an essential component of the incorporation of the explants (14, 21, 31, 32). On the other side, calcium comes with some disadvantages like the precipitation of calcium salts, reduction in the cell division process, and interruption of synthetic seed formation and growth (9). So, other cations like magnesium, barium and mixed cations can also be taken as possible candidates that can improve the performance of artificial seeds. The optimal concentration of cations for artificial seed production is from 50 mM to 200 mM (13, 19). Though the specific effects of magnesium and mixed cations were not directly addressed in the context of synthetic seeds, but the effect of various cations is studied on germination and plant growth. In hydroponic systems, the production of seed potatoes is enhanced by magnesium (Mg), whose proper dose is essential for plant metabolism and chlorophyll formation (33). However, excessive Mg can be detrimental, causing seedling abnormalities (34). When it comes to seed germination and seedling growth, different salts have distinct impacts (34).

### The pH of the solution

The pH of the solution determines the gelation and the integrity of the matrix, as well as the viability and differentiation of the shoots. The pH of the medium will change the ionizations as well as the solubility of the entrapping agents; on the other hand, the availability and the activity of the cations will be affected. Unstable gel is the product of an acidic environment, as it cannot withstand during the process of its storage or handling. A pH of 6.6 can therefore result in a structurally sound gel, which can help prevent degradation and contamination (35). Nevertheless, a high pH can have accumulative effects on the viability or even impede the development of the explants, as well as alter the cell membrane permeability, enzymatic activity, and the metabolism of the cells. This is why a neutral or mildly acidic pH should be set for synthetic seed synthesis, depending on the plant species as well as the type of explants (35). Between pH 5 and pH 7.5, synthetic seed production will be optimally fulfilled by encapsulation agents depending on their type and the plant species (13, 19). Though the impact of pH on synthetic seeds has not been well studied, the appropriate study can be done to examine the impact.

### *The osmotic potential of the solution*

The osmotic potential of the solution is a factor that influences the water content, water loss and success of the culture, and the growth and survival of the explants. The osmotic potential of the solution is decided by the solute concentration, which can consist of sugars, salts, or polyols, which further strengthen the matrix. High osmotic potential can cause the low water content of the matrix together with the high water loss, which consequently produces protection from microbial infections and the conditions of desiccation tolerance of the explants (36). Nevertheless, a high status of the plant cell in the osmotic potential may also negatively affect the developmental activities and life ability of the explants, with the possible consequences of plasmolysis, osmotic stress, and metabolic inhibition of the cells. Accordingly, osmotic potential of very low value must be supported for the production of synthetic seeds for various plants and the conducting of experimental works on the use of different explants (36). The optimal osmotic potential of the environment in which synthetic seed should be produced is in the range of -0.5 MPa to -1.5 MPa depending on the type and concentration of solutes, and the specific plant species (13,19).

### *The presence of additives*

The presence of additives may help to improve the qualities of the matrix and, relatedly, the development of the separated explants. Various types of additives could be included in the matrix, like activated charcoal, polyethylene glycol, sucrose, amino acids, vitamins, hormones, antibiotics and fungicides, among others, depending on the plant species and the type of explants. The composition of this matrix significantly influences seed formation, germination and subsequent plant growth. Sodium alginate is commonly used as the encapsulation material, with its concentration affecting seed texture and germination rates (26). Activated charcoal may provide the optical properties and the stability of a bio-composite matrix through its role of absorbing impurities and oxidation prevention (29). Polyethylene glycol, which is a liquid poly-

mer, can be used to optimize the mechanical strength and the permeability of the matrix with an increment in the viscosity and the porosity of the gel. It has shown positive effects on somatic embryo maturation in various species, including hybrid fir (37) and papaya (38). Sucrose can have an impact on the osmotic potential and the availability of nutrients in the medium, where it acts as the source of carbon and energy substances for the explants. Studies have shown that incorporating nutrients, growth regulators, and additives like activated charcoal into the matrix can enhance germination and plant development (29, 39). Sucrose concentration in the medium plays a crucial role, with 3% often yielding optimal results for germination and growth (40). Proteins, vitamins, hormones, antibiotics, and fungicides can be used to effect growth and development through elements supplied and inhibition of microbial infection (13, 19). The addition of growth regulators like benzyl adenine (BA) and nutrients to the alginate matrix can enhance germination rates (26, 29). However, excessive concentrations of growth regulators may inhibit development (41).

The matrix formulation for synthetic seed-making depends on the plant type, explant type, and encapsulating agent type. The synthetic seed matrix may produce varying effects on the seed quality and performance with different compositions of the matrix. Consequently, cultivating conditions for individual products and each plant species should be optimized so that we can reap the maximum yield. While synthetic seed technology offers potential for large-scale propagation and germplasm conservation, challenges remain, including limited availability of suitable micro propagules and low conversion rates of synthetic seeds into normal plants (19).

### *Advantages and limitations*

Synthetic seed technology has several advantages and limitations for medicinal plants, which are discussed below (41, 42).

#### Advantages

- Synthetic seed technology will help to propagate the medicinal plants at large by producing the exact uniform and disease-free plants on mass in a short period and at a low cost. The synthetic seed technology is capable of addressing several issues, ranging from low seedling viability, poor seed germination rate, and slow seed multiplication to seasonal dependence, which is evident in dependence on conventional vegetation reproduction.
- Synthesized seed technology can make its way into the germplasm of medicinal plant storage, by creating a handy and effective way of plant material stock and transportation. Besides, synthetic seeds that can prevent genetic erosion and minimize the metabolic content of medicinal plants can also be achieved via copying or close to the genotype and phenotype of the explants. Synthetic seeds can also be combined with cryopreservation, which can work to maintain the shelf life and the stability of synthetic seeds and their disintegration.
- The technological advance of synthetic seeds towards medicinal plants can be developed through somatic hybridization, gene transfer or mutagenesis by making an introduction of vital traits. Transgenic seeds can also serve as a transport system for gene transfer, by transporting, for example, modified cells or organs. Synthetic seeds are being utilized as a measure for studying the genetic formation of medicinal plants to offer scientists a data set that is uniform and controllable.

#### Limitations

- The tech-free seed technology, however, also falls short of a few points, including the inconsistency in the conversion and germination rates, which can also lead to the reduced reliability of synthetic seed production. The germination and conversion rates of synthetic seeds are impacted by several factors, for example, the type of explants, the encapsulating agent, the composition of the matrix, storage conditions, germination conditions, and so on. Thus, it is required to optimise the protocols for each plant or species and different types of synthetic seeds for better results.
- Another issue that is widely recognized with synthetic seed technology is generally connected with the issue of microbial contamination, which can consequently affect the quality and the performance of synthetic seeds. Microbial contamination is a possible source of infection during either the encapsulation process or storage period, and there could be the deteriorating germination stage, which leads to the deterioration, the infection, or the death of synthetic seeds. Hence, adapting sterile methods and properly adding enhancements such as anti-microbial agents or fungicides would wipe out the presence of harmful microorganisms.
- Synthetic seed technology presents certain challenges because the generalization and optimization of protocols directly impact the cost and the reliability of synthetic seed production. Standardizing and validating protocols for synthetic seed production requires plenty of resources and time, which may differ in numerous cases like types of media and explants, encapsulating agents and matrix composition. So, extensive research and development work is necessary to formulate such protocols.

### *Future perspectives*

Synthetic seed technology has the upper hand in seed production technology, it is capable of overcoming the bottlenecks witnessed in traditional ways and introducing new dimensions in the mass propagation, preservation of germplasm and improvement of genetics. The downside of synthetic seed engineering involves variability in germination rates and conversion rates (1, 41), the possibility of microbial contamination (43), and the requirement for

innovative protocol optimization. Therefore, some suggestions for future research and development of synthetic seed technology for medicinal plants are as follows:

### Improvement of germination and conversion rates

The major problem associated with the adoption of the new technology of synthetic seed is low germination success, also known as the conversion rate of synthetic seeds, which may affect the efficiency and reliability of especially the process of production. Developing somatic embryos is a highly unsynchronised process in *T. polium*. There are also other factors like plant tissue source, storage method, and storage time that affect the germination or conversion rates (44). Thus, dealing with their germination and conversion rates is necessary, by enhancing such factors that affect them like the explant type, encapsulation material, matrix composition, storage conditions, germination conditions etc, and through the improvement of other approaches like picking the best priming agents and growth regulators etc (11). So, there is a need to develop and standardize proper methods to increase the germination and conversion rates in synthetic seeds.

### Enhancement of genetic stability

Despite all the advantages, a main problem in artificial seed technology could be the introduction of genetic disturbance or soma clonal variation in artificial seeds, which in turn influence the quality and the performance of artificial seeds (45). As the genetic stability is still not known in many reports except for some species such as *Rauvolfia tetraphylla* (46), *Rauvolfia serpentina* (47, 48), *Stevia rebaudiana* (49) etc. Though the methods have shown high genetic stability in plantlets derived from synthetic seeds, some minor variations can also be seen (50). Thus, the genetic stability of synthetic seeds can be strengthened by minimizing the factors leading to soma clonal variation, which could include duration and type of culture medium, plant growth regulators, encapsulating agent as well as others. This is accompanied by the monitoring and evaluation of the genetic stability of synthetic seeds using lab molecular markers, e.g. split analysis methods such as RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeats), SSR (Simple Sequence Repeats) etc (11). For the use of synthetic seeds in the conservation and propagation of endangered species, the protocols need to be standardized to improve the genetic stability over a long period, as there is no proper research on the long-term storage of synthetic seeds.

### Incorporation of secondary metabolites

These bioactive compounds have medicinal value and are used in drug development worldwide (51, 52). The primary importance of the synthetic seed technology is the preservation of secondary metabolites of medicinal plants, which are vital for their catalysis of therapeutic applications. *In vitro* culture methods offer advantages over conventional cultivation, including controlled environmental conditions and the ability to enhance metabolite production using elicitors (53). However, the secondary metabolite content of synthetic seeds may vary depending on the type of explants, the type of encapsulating agent, the matrix composition, the storage conditions, the germination conditions etc. Therefore, it is necessary to incorporate the secondary metabolites into synthetic seeds, by optimizing the factors that affect their biosynthesis, such as the culture medium, the plant growth regulators, the precursors etc. Like elicitors, which are the small molecules that trigger plant secondary metabolite production via modulation and activation of many biosynthetic pathways and signalling cascades (54) can be applied to various mediums such as MS (Murashige and Skoog), and B5 for enhanced production of secondary metabolites (55). Methyl-jasmonate and salicylic acid are potent elicitors that can stimulate secondary metabolite synthesis in various medicinal plants (51, 52). It is also necessary to measure and analyze the secondary metabolite content of synthetic seeds, by using analytical techniques, such as HPLC (High Performance Liquid Chromatography), GC-MS (Gas Chromatography -Mass Spectrometry) and NMR (Nuclear Magnetic Resonance) etc (11).

### Integration of cryopreservation

Cryopreservation, using liquid nitrogen at -196°C, is a safe and cost-effective method for long-term conservation of plant genetic resources (56). The choice of appropriate cryopreservation technique for various types of explants primarily depends on the physiological state of the cells undertaken (57, 58). One key advantage of this technology is the opportunity to preserve and transport seeds into storage or even bring them to remote places, which is necessary for plant germplasm conservation. However, it should be considered that the cold storage and transport of synthetic seeds may need special conditions, such as low temperature to preserve synthetic seeds and some constraints like low humidity or low oxygen etc., which may affect the multiplication and development of the seeds. The successful preservation of shoot tips at -196°C allows for long-term storage without loss of genetic integrity, which is crucial for maintaining plant biodiversity (59). The success of cryopreservation and synthetic seed relies on various factors such as explant type, time of application, osmotic regulators, and other chemicals used in the process (57). Besides, cryopreservation is thought to be one of the most beneficial procedures for synthetic seeds by lifting germination rates, conversion of the explant, and stabilization of their genetics (11). Continuous research is expected to optimize protocols, broader application, and application towards a greater number of plant species.

### Evaluation of field performance

Another major objective of synthetic seed technology is the creation of plants that have identical design features to their original, ranging from their physical and physiological make-up to their biochemistry as well as their genetic constitution. Though synthetic seeds are now tested for higher germination and conversion rates (60, 61) and can be stored for upto 150 days in some cases (60), the real challenge lies in the practical implications of such seeds. Although the field performance of such seeds is being eval-

uated and, in some cases, no significant difference is observed (62, 63), it is necessary to evaluate the field performance in a wider range of crops and factors that affect the field performance of the synthetic seeds. The real performance of artificial seeds in the field might vary from the test results due to the surrounding factors such as the light, temperature, moisture, soil etc. Therefore, the field performance of artificial seeds should not be just compared with the regular plants in terms of their growth, development, yield, and quality but also environmental issues of artificial seeds should be assessed, particularly their interaction with the biotic and abiotic elements such as pest and diseases (11).

### Development of disease-free synthetic seeds

The need for disease-free synthetic seeds is very important due to the chance of pathogen infection in synthetic seeds (43). In recent studies, seeds produced in sterile and aseptic conditions showed healthy growth and prevented any type of contamination (18, 43). There is a need to develop other methods and standardise existing ones for the development of disease-free synthetic seeds.

Synthetic seed technology is a very promising and unique method that can be used for the conservation of medicinal plants as well as it can be utilized to offer varieties of advantages in comparison with the usual methodology. Although the technology of synthetic seeds is developing, there is still the need for more of these new seeds to be tested and improved to make output more effective and reliable. Synthetic seed technology could become the way out towards improved methods of multiplication and preservation of medicinal plants and a key to their wider application and sustainable and profit-driven utilization. Tools like synthetic seeds have the potential to increase the crop propagation rate, and propagation of endangered species plays an important role in germplasm preservation (64).

## Conclusion

Synthetic seeds have the potential to replace the conventional ones in mass propagation, preservation, and genetic modification of medicinal plants. The artificial seed technology is improving the production of uniform and disease -free plants, cutting down the cultivation time and cost, maintaining genetic diversity, and facilitating global exchange of germplasm.

Although the application of synthetic seed technology results in numerous benefits, it also brings some constraints, like fluctuations in germination and conversion rates, microbial contamination, and refinement of protocols. Additionally, it has been questioned if cryopreservation methods have an impact on genetic stability, but there isn't any concrete evidence to back this up. Hence, synthetic seed technology entails more in-depth research and development to overcome the challenges and hurdles and make synthetic seed production a reliable and efficient process. Synthetically produced seeds can open the universe of conservation and utilization of various plants and can also help in the sustainable and profitable use of these plants.

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## Authors' contributions

SS carried out the entire study and participated in preparing the manuscript. SPM participated in the collection of material and drafted the manuscript and coordination. SJ participated in the collection of material. SK and SSP participated in the alignment. SKS provided the overall idea about the topic and preparation.

## Compliance with ethical standards

**Conflict of interest**: Authors do not have any conflict of interests to declare.

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