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# Synthetic Seed Production; its Relevance and Future Panorama

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## ABSTRACT

The synthetic seed acquaintance has been developed to use somatic embryos and/or other micropropagules as seed analogues successfully in the field or greenhouse, and their mechanical planting at a mercantile level. Synthetic seed development from somatic embryo opens up new vistas in the field of agriculture. The technology provides methods for preparation of seed analogues called synthetic seeds or artificial seeds from the micropropagules like somatic embryos, axillary shoot buds, apical shoot tips, embryogenic calli as well as protocorm or protocorm like bodies. These artificial seeds offer an important packaging system. The technique cut short lengthy choice procedure of the usual recombination breeding and can convey the advancements of biotechnology to the doorsteps of the farmer in a cost-effective manner. Synthetic seeds present a number of return, easy management, storability, compact size of propagules, and transportability. This review provided useful information for the production and utilisation of synthetic seed through encapsulation of differentiating propagules (tissue fragments with shoot primordia) for various species. The present review focuses on the technology developed, its achievements, current scenario, the limitations resisting the application of the synthetic seed technology and the future perspectives.

**Keywords**: Synthetic seed, Artificial seed, Micropropagation, Explants

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## INTRODUCTION

Any artificially encapsulated somatic embryo, shoot bud, cell aggregate, or any other plant tissue that can be used for sowing as a seed, competent of converting into a plant under *in vitro* or *ex vitro* conditions, and retaining this proficiency even after storage is referred to as synthetic or artificial seed. Earlier, somatic embryos that were of economic use in crop production and plant delivery to the field or green house were termed as artificial seeds[1]. However, recently it was found that other micropropagules like shoot buds shoot tips, organogenic or embryogenic calli, etc. can also be employed for the production of synthetic seeds[2]. Thus, the border line of synthetic seeds has been widened to the fields of somatic embryogenesis, besides encompassing other techniques of micropropagation like organogenesis and enhanced axillary bud proliferation system[3]. According to Murashige synthetic seed is "an encapsulated single somatic embryo" a clonal product that could be handled and used as a true seed for transport, storage, and sowing, and that, therefore, would eventually grow, either in vivo or ex vitro, into a plantlet[4]. Synthetic seeds act as low-cost-high volume propagation system.

Implementation of synthetic seed technology requires manipulation of *in vitro* culture systems for large scale production of viable materials, which are able to convert into plants, for encapsulation. Through these systems as they are potent structures for plant regeneration either after having minor treatment or without any treatment with growth regulators[5]. Developments in tissue culture have bewilderingly paved the way for the mass production of somatic embryos by various techniques like somatic embryogenesis, organogenesis and enhanced axillary bud proliferation systems which could be used for encapsulation[6]. Because the naked micropropagules are sensitive to desiccation and or pathogens when exposed to natural environment, it is envisaged that for large scale mechanical planting and to develop the success of plant delivery to the field or greenhouse, the somatic embryos or even the other micropropagules useful in synthetic seed production would necessarily require some protective coatings[7]. The encapsulation technology has been applied to produce synthetic seeds of a number of plant species[8]. Encapsulation is accepted to be the best technique to offer shield and to convert the *in vitro* derived propagules into ‘synthetic seeds’ or ‘synseeds’ or ‘artificial seeds[9]. Fabrication of artificial seeds has unraveled new gift in plant biotechnology[10]. The synthetic seed technology is considered to mingle with the advantages of clonal propagation with those of seed propagation and storage[11]. It is a time and hand labor saving procedure for the use of direct organogenesis in the production of shoot tips or shoots clusters suitable for encapsulation[12]. An

*in vitro* derived explants would be enclose inside a nutritive and protective capsule of synseed, easily storing them in a limited space. The artificial seeds provide an important packaging system[13]. Each unit of the synthetic seed give plantlet after sowing in appropriate conditions[14]. According to Bapat, this technique combine the compensation of cloning and those of the true seed as far as delivery, conservation and manipulation is concerned. The artificial seed technology has added new dimensions for handling, transplantations and mass propagation of plants[15]. It plays a wonderful role for conservation of endangered and desirable genotypes[16].

**Difference between natural and artificial seed**

**a) Plant Seeds**

A plant seed consists of an embryo and its stored food (endosperm), surrounded by a seed coat (testa).

1. Embryo

The embryo is made up of one or two cotyledons attached to a central axis. The upper part of the axis contains a plumule at its tip which grows into the shoot system. The lower part of the axis consists of the hypocotyl and a radicle. The radicle grows into the root system[17-19].

2. Endosperm

The endosperm is the food reserve used by the embryo during the early stages of germination. Before the embryo is able to perform photosynthesis, the endosperm provides vital nutrients to the embryo[20].

3. Testa

The testa is the covering of the seed which protects the embryo from injury and drying out and also ensures the viability of the embryo before germination[21]. During germination occurs the seed coat breaks following the water adsorption, allowing the radicle to first emerge from the seed[22-23].

**b) Artificial Seeds**

1. Artificial seeds can be produced by encapsulating a plant propagule in a matrix which allows it to grow into a plant[24].
2. The growth of the plants can be controlled by the chemicals used in the nutrient media[25-26].
3. In artificial seeds, an artificial endosperm can be created within the encapsulation matrix[27].
4. The encapsulation matrix is a hydrogel of natural extracts from seaweed (agar, carageenan or alginate), plants (arabic or tragacanth), seed gums (guar, locust bean gum

or tamarind) or microorganisms (dextran, gellan or xanthan gum)[28].

5. Useful adjuvants such as nutrients, plant growth regulators, pesticides and fungicides can be furnished to the plant propagule within the encapsulation matrix[29-30].

**Production of artificial seeds**

Seeds are the delivery systems for agricultural biotechnology. High quality seed leads to indispensable seedling performance in the field[31-32]. It is the ultimate basis of successful companies that breed crop plants for seed production. Seed quality is a complex trait, ascertained by the interactions between the multiple genetic factors and the environmental conditions[33]. Modern slants to perk up the seed quality therefore coalesces classical genetics, omics approaches and a variety of seed technologies[34]. These "seed biotechnologies" augment the physiological quality, and vigour to establish a crop in the field under diverse environmental conditions. Production or the construction of artificial seeds has tattered novel vistas in plant biotechnology[35]. Production of synthetic seeds has been successfully accomplished in a number of naturally and other commercially important plants like soybean, mustards, coffee, tobacco, and cotton[36].

**ARTIFICIAL SEED PRODUCTION METHODS**

Synthetic seed production can be carried out by two main methods;

**Desiccated system for artificial seed production**

In this method, somatic embryos are first acclimatized to overcome desiccation prior to encapsulation. Then these hardened embryos are encapsulated artificially with the use of suitable growth medium. Synthetic seeds were developed by using desiccation technique for the encapsulation of carrot somatic embryos[37]. For encapsulation of embryos, they selected polyoxyethylene for being readily soluble in water, forming a thin film. Besides this polyoxyethylene does not support the growth of micro-organisms and is not toxic to the embryo. Jung in 2004 formulated a method for incapsulation of carrot somatic embryos and callus in polyoxyethylene glycol[38]. The coating mixture is allowed to dry for several hours on a teflon surface in a sterile hood. The dried mixture is then placed on a culture medium and allowed to rehydrate for embryo survival[39]. The desiccated synthetic seeds are produced by encapsulation of somatic embryos in polyoxyethylene glycol[40]. A period of one or two weeks are required for desiccation by using a chamber with relatively low humidity, or by unsealing the petridishes rapidly and leaving them on the bench overnight to dry up[41]. This method is most reliable for desiccation tolerant plant species[42].

**Hydrated system for artificial seed production**

This method is commonly exercised for somatic embryos of plant species which are sensitive to desiccation. The somatic embryos are encapsulated in the form of hydrogel or hydrogel capsules[43]. The gel used to enclose somatic embryo remains hydrated. A variety of water soluble gels can be used for the purpose of encapsulation, like alginate, gel rite, locust bean gum, sodium alginate with gelatine. However, alginate has been found to be the most apposite gel. Redenbaugh in 1986 imparted a method for hydrogel encapsulation of individual somatic embryos of alfalfa[44]. Since then encapsulation in hydrogel remains to be the most premeditated method of artificial seed production[45].

**Encapsulating agents for artificial seed production**

Various encapsulating agents have been tried, out of which agar, agarose, alginate, carragenan, gums, dextrans, gelrite, and polyacrylamide are noteworthy. Besides several gelling agent like nitrocellulose, ethylocellulose, carboxymethyl cellulose, polyco-2133, sodium pectate, tragacanth gum have also been tried for encapsulation of synthetic seed. Of all these alginate encapsulation was found to be more appropriate and feasible for synthetic seed production[46]. Alginate hydrogel is often preferred as a matrix for synthetic seed because of its moderate viscosity, low toxicity for somatic embryos, quick gelation, low cost, biocompatibility characteristics, its solubility at room temperature and its ability to form completely permeable gel with calcium chloride. Beside routinely practice of agar as gelling agent, its use is deliberately avoided as it is comparatively inferior to alginate with respect to long term storage. Alginate is selected because it enhances capsule formation and also the rigidity of alginate beads provided better protection than agar to the encased somatic embryos against mechanical injuries. Sodium alginate is also most suitable encapsulating agent for handling and storage of orchids like *Geodorum densiflorum*, *Dendrobium wardianum*, *Phaius tankervillae*, and *Spathoglottis plicata*. Structurally alginate is a straight chain, hydrophlic, colloidalpolyuronic acid primarily composed of hydro-*β*-D-mannuronic acid residues with 1-4 linkage. The basic principle entailed in the alginate encapsulation practice is that the somatic embryos are mixed with sodium alginate gel (0.5– 5.0% w/v) and dropped into a calcium salt solution [$CaCl_2$ (30–100 mM), Ca $(NO_3)_2$ (30–100 mM)] where ion-exchange reaction occurs and sodium ions are replaced by calcium ions forming calcium alginate beads or capsules surrounding the somatic embryos. The size of the capsule is controlled by varying the inner diameter of the pipette nozzle. Exchange of sodium ions with calcium ions is responsible for hardness and stiffness of the capsule as well as the duration of complexing. Hence the concentration of the two gelling agents, sodium alginate and $CaCl_2.2H_2O$, and the complexing time should be optimized. In general 3% sodium alginate upon

complexation with 75Mm $CaCl_2.2H_2O$ for half an hour gives optimum bead hardness and rigidity for the production of viable synthetic seed. However, Lisek and Orlikowska have revealed that for the production of synthetic seeds of carrot, 1% sodium alginate solution, 50 mM $Ca^{2+}$ and 20–30 min time period is reasonable for proper hardening of calcium alginate capsules. They have suggested the use of a dual nozzle pipette in which the embryos flow through the inner pipette and the alginate solution through the outer pipette. Consequently, the embryos are positioned in the centre of the beads for better protection[47].

**METHODS FOR ARTIFICIAL SEED ENCAPSULATION:**

The two main methods adopted for the artificial seed encapsulation are;

**Dropping method**

This method involves the dipping of somatic embryos in the hydrogel, which encases these embryos. Different materials like Alginate – sodium alginate, agar from see weeds Seed gums like guar gum, locust bean gum can be used for the formulation of hydrogel[48]. However sodium alginate solution (1–5%), prepared in MS basal medium solution is mostly preferred. After dipping the somatic embryos in the sodium alginate solution, the coated beads are added one by one with the help of pipette (5mm) into a complexation solution (100mM calcium nitrate solution) flask kept on magnetic stirrer and kept as such for about 20-30 minutes[49]. Embryos get covered by stably complexed calcium which further becomes harder. After hardening the gelled embryos are washed with either water or MS basal medium after which the synthetic seeds are ready. Alternatively, a burette is filled with sodium alginate solution (1 – 5%), dripped into a calcium nitrate solution (100mM) drop by drop. Somatic embryo is inserted into the drop formed at the burette tip. Sodium alginate drop along with SE falls into the solution of calcium nitrate. Additionally expedient adjuvants like growth regulators, herbicides, insecticides, fungicides and mycorrhizae can be delivered to the SE while encapsulation along with the matrix. This approach is applicable for embryo / auxiliary / apical / adventitious buds[50].

**Molding method**

This process follows easy procedure of mixing of embryos with temperature dependent gel (e.g. gel rite, agar). Cells get coated with the gel during lowering of the temperature.

**Nourishment for zygotic embryos:**

As the testa and endosperm which is present in the natural seeds are absent in somatic embryos, the artificial seeds fall short of protection and food deficiency[51]. To combat with these deficiencies, addendum of nutrients and growth regulators to the encapsulation matrix is done, which serve as an artificial endosperm thus helping in the germination and maintenance of

viability of the encapsulated somatic embryos. The artificial seeds can then easily be stored at $4^{\circ}C$[52].

**Protection of encapsulated somatic embryos:**

To prevent the embryos from dehydration and mechanical injury, a number of worthwhile resources such as nutrients, fungicide, pesticides, antibiotics and rhizobia may be included into the encapsulation matrix [53]. Merging of activated charcoal perks up the conservation and vitality of the encapsulated somatic embryos, by augmenting the respiration of somatic embryos and thus splitting up the alginate. In addition, charcoal stores nutrients within the hydrogel capsule and gradually makes them available for the growing embryos[54].

**Important strategies to improve seed and seedling performance**s:

The methods to enhance seed and seedling performance are through addition of chemicals to protect the seed from pathogens and/or to improve germination[55]. Different techniques like film-coating, seed coating and seed pelleting are used for this.

**Film-coating:**

This method allows the chemicals to be applied in a synthetic polymer that is sprayed onto the seeds and provide a solid, thin coat covering them. The advantage of the polymers is that they adhere tightly to the seed and prevent loss of active materials like fungicides, nutrients, colorants or plant hormones. Some novel applications of film coating are used to modify imbibition and germination. They can confer temperature-sensitive water permeability to seeds or affect gaseous exchange. By this they control the timing of seed germination and seedling emergence[56].

**Seed pelleting:**

This provide a thicker artificial coverings to seeds, which can be used to cover irregular seed shapes and add chemicals to the pellet matrix, e.g. of sugar beet or vegetable seeds. The pellet matrix consists of filling materials and glue. Loam, starch, tyllose (cellulose derivative) or polyacrylate/polyacrylamide polymers are commercially used[57]. Seed pelleting is also used to increase the size of very small horticultural seeds. This provides improved planting features, e.g. singulate planting, the use of planting machines, or precise placement and visibility in/on the soil [58-59].

**Seed priming and pre-germinated seed:**

Seed priming is the most important physiological seed enhancement method. Seed priming is a hydration treatment that allows controlled imbibition and induction of the pregerminative metabolism. This leads to better crop and higher yields. A practical drawback of primed seeds is often a decrease in storability and the need for cool storage temperatures[60]. There are different

methods of seed priming. **Osmopriming** (osmo-conditioning) is the standard priming technique. Seeds are incubated in well aerated solutions with a low water potential, and afterwards washes and dried[61]. The low water potential of the solutions can be achieved by adding osmotica like mannitol, polyethyleneglycol (PEG) or salts like KCl[62]. **Hydropriming** (drum priming) is achieved by continuous or successive addition of a limited amount of water to the seeds[63]. A drum is used for this purpose and the water can also be applied by humid air. 'On-farm steeping' is the cheep and useful technique that is practiced by incubating seeds (cereals, legumes) for a limited time in warm water[64]. **Matrixpriming** (matriconditioning) is the incubation of seeds in a solid, insoluble matrix (vermiculite, diatomaceous earth, cross-linked highly water-absorbent polymers) with a limited amount of water. This method confers a slow imbibitions[65]. **Pregerminated seeds** is only possible with a few species. In contrast to normal priming, seeds are allowed to perform radicle protrusion. This is followed by sorting for specific stages, a treatment that re-induces dessication tolerance, and drying. The use of pregerminated seeds causes rapid and uniform seedling development[66].

**ADVANTAGES OF SYNTHETIC SEEDS:**

Utilization of synthetic seeds appears to be particularly promising. The encapsulation, storage and re-growth of homogeneous material permit the prospect of automated mass production of selected plant species. There are a number of prospective uses of artificial seeds of those crop plants that are vegetatively propagated and have long juvenile periods, e.g. citrus, grapes, mango, etc. The synthetic seed would also be a channel for new plant line produced through the biotechnological advances to be delivered directly to the green house or field. It is a high volume, low cost production technology. Synthetic seeds have been found highly advantageous for germplasm conservation in grape and other similar crops.

**Easy handling**–During storage, transportation and planting, as these are of small size.

**Inexpensive transport** – Reason behind is small size. Not a season dependent technology.

**Storage life** – Much longer, seed viability remains good for longer time period.

**Product uniformity** – As somatic embryos used are genetically identical.

**Conservation of endangered species** – In Hedgehog cacti (Echinocereus sp.)

**Large scale propagation** – Very much suitable for large scale monoculture.

**Mixed genotype plantations** – Suitable for this too, as for monoculture.

**Germplasm conservation** – Important in germplasm conservation.

**Elite plant genotypes** – Artificial Seed Technology preserves / protects and permits economical mass propagation of elite plant genotypes.

**Permits direct field use** – Rooting, hardening is necessary as it is in tissue culture plants. It permits direct field sowing.

**Facilitates study** of seed coat formation, function of endosperm in embryo development and seed germination, somaclonal variation.

**Supply of beneficial adjuvants** – Beneficial adjuvants like plant nutrients, plant growth regulators, microorganisms, fungicides, mycorrhizae, antibiotics can be made available to thedeveloping plant embryo as per the requirement as these can be added in to the matrix.

**Propagation** of plants unable to produce viable seeds.

**Hybrid production** – Synthetic Seed Production technology can be used for production of hybrids which have unstable genotypes or show seed sterility. It can be used in combination with embryo rescue technique. The rescued embryo can be encapsulated with this technique.

**Limitations**

In spite of the fact that the technology offers wonderful and rapidly emergent area of research in plant cell, micropropagation and tissue culture, it still has limitations for commercial and practical uses. The plantlet production rates by the micropropagated are not always satisfactory and not warrant high investments. Regarding this set of problems, adventitious shoot proliferation might prove to be a more productive pathway than axillary shoot proliferation, which is the most commonly used method in commercial labs[67]. Adventitious shoot proliferation from leaf blades has demonstrated high productive potential in woody species[68]. Secondly another problem related to the outlay of this technology is that the ending product is represented by small plantlets, which require definite and costly management until commercialization and final delivery[69].

**Achievements and Future prospects**

The judicious and intelligent coupling of artificial seed technology with that of microcomputer in achieving automated encapsulation and regeneration of plantlets would tremendously increase the efficiency of encapsulation and production of homogeneous and high quality artificial seeds, and will thus revolutionize the current concept of commercial micropropagation method by the beginning of twenty-first century[70].The article provided useful information for the production of synthetic seed through encapsulation of differentiating propagules (tissue fragments with shoot primordia) in various species. Somatic embryos are bipolar structure with apical and basal meristematic regions, which are capable of forming shoot and root, respectively. A plant derived

from a somatic embryo is some time referred to as 'embling'. While various micropropagules have been considered for synthetic seed production, the somatic embryos have been largely favoured as these structures acquire the radicle and plumule that are capable to expand into root and shoot in one step, usually without any definite handling. Preparations of artificial seeds by the use of somatic embryos are increasing in a number of plant species as is becoming more feasible in tissue culture technology Table 1. Various types of artificial seeds have been prepared using somatic embryos which have been either dried or maintained fully hydrated, these may or may not be encapsulated. Somatic embryo has the additional advantage of serving as a germplasm storage system, it maintains propagule in a inert state for extensive periods of time. Dried somatic embryos cut short the space and labour in a commercial production system and increasing the storage for planting in the future.

**Table 1. Plant species in which encapsulation technology has been applied for the production of synthetic seeds**

| Name of Plant | Family | Common Name | Part use | References |
|---|---|---|---|---|
| *Medicago sativa* | Fabaceae | Alfalfa | SEs | 33 |
| *Arachis hypogaea* | Fabaceae | Groundnut | SEs | 36 |
| *Crataegus oxyacantha* | Rosaceae | Hawthorn | SBs | 71 |
| *Mangifera indica* | Anacariaceae | Mango | SEs | 72 |
| *Psidium guajava* | Myrtaceae | Guava | SEs | 73 |
| *Eucalyptus citriodora* | Myrtaceae | Eucalyptus | SEs | 74 |
| *Malus pumila* | Rosaceae | Apple rootstock | SBs | 75 |
| *Rubus idaeus* | Rosaceae | Raspberry | SBs | 76 |
| *Dendrobium wardianum* | Orchidaceae | Orchid | PLBs | 77 |
| *Betula pendula* | Betulaceae | Birch | SBs | 78 |
| *Cthanranthus roseus* | Apocynaceae | Sadabahar | SEs | 80 |

SEs, somatic embryos, SBs, shoot buds; ABs, axillary buds; EMs,embryogenicmasses; PL protocorm-like bodies.

## CONCLUSION

The requirement for the practical application of the artificial seed technology is the large-scale production of high quality micropropagules, which is at present a major limiting factor. The synthetic seed technology offers tremendous potential in micropropagation and germplasm conservation; however further research is needed to perfect the technology so that it can be used on a commercial scale. Recent advance in the production of artificial seeds reveal that beside somatic embryos, encapsulation of cells and tissues developed *in vitro* is becoming popular. It offers a simple way of handling cell and tissues, protecting them against strong external gradients and as an efficient delivery system.

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