Synthetic seed: Prospects and limitations

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The synthetic seed technology 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 commercial level. 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. For the last fifteen years, intensive research efforts have been made on synthetic seed production in a number of plant species. Despite these researches, practical implementation of the technology is yet to be fully realized due to limitations encountered with the production, development, maturation and subsequent conversion of the micropropagules into plantlets under *in vitro* or *ex vitro* conditions. The present article focuses on the technology developed, its achievements and prospects as well as limitations resisting the application of the synthetic seed technology.

SYNTHETIC seeds are defined as artificially encapsulated somatic embryos, shoot buds, cell aggregates, or any other tissue that can be used for sowing as a seed and that possess the ability to convert into a plant under *in vitro* or *ex vitro* conditions, and that retain this potential also after storage[1]. Earlier, synthetic seeds were referred only to the somatic embryos that were of economic use in crop production and plant delivery to the field or greenhouse[2,3]. In the recent past, however, other micropropagules like shoot buds, shoot tips, organogenic or embryogenic calli, etc. have also been employed in the production of synthetic seeds. Thus, the concept of synthetic seeds has been set free from its bonds to somatic embryogenesis, and links the term not only to its use (storage and sowing) and product (plantlet) but also to other techniques of micropropagation like organogenesis and enhanced axillary bud proliferation system.

Implementation of synthetic seed technology requires manipulation of *in vitro* culture systems for large-scale production of viable materials, that are able to convert into plants, for encapsulation. Somatic embryogenesis, organogenesis and enhanced axillary bud proliferation systems are the efficient techniques for rapid and large-scale *in vitro* multiplication of elite and desirable plant species. Through these systems a large number of somatic embryos or shoot buds are produced which are used as efficient planting material as they are potent structures for plant regeneration either after having minor treatment or without any treatment with growth regulator(s). 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 improve the success of plant (*in vitro* derived) 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. Encapsulation is expected to be the best method to provide protection and to convert the *in vitro* derived propagules into 'synthetic seeds' or 'synseeds' or 'artificial seeds'[4,5]. The encapsulation technology has been applied to produce synthetic seeds of a number of plant species belonging to angiosperms and gymnosperms (Table 1). Nevertheless, their number is quite small in comparison to the total number of plant species in which *in vitro* regeneration system has been established.

Production of artificial seeds has unravelled new vistas in plant biotechnology. The synthetic seed technology is designed to combine the advantages of clonal propagation with those of seed propagation and storage. Despite the fact that the technology is an exciting and rapidly growing area of research in plant cell and tissue culture, there are many limitations for its practical use. The purpose of this review is to present a report on prospects and limitations of synthetic seed production. The subject has been earlier reviewed in a different context by various researchers[3,5–7].

The technology

Basic hindrance to synthetic seed technology was primarily based on the fact that the somatic embryos lack important accessory tissues, i.e. *endosperm* and *protective coatings*, that make them inconvenient to store and handle[5]. Furthermore, they are generally regarded to lack a quiescent resting phase and to be incapable of undergoing dehydration. The primary goal of synthetic seed research was, therefore, to produce somatic em-

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bryos that resemble more closely the seed embryos in storage and handling characteristics so that they can be utilized as a unit for clonal plant propagation and germplasm conservation. In achieving such a goal the technology of encapsulation has evolved as the first major step for production of synthetic seeds. Later it was thought that the encapsulated synthetic seed should also contain growth nutrients, plant growth promoting micro-organisms (e.g. mycorrhizae), and/or other biological components necessary for optimal embryo-to-plant development. A number of patents covering the development of seed analogues have been issued[7]. However, success of the synthetic seed technology is constrained due to scarcity and undesirable qualities of somatic embryos making it difficult for their development into plants. The choice of coating material for making synseeds is also an important aspect for synseed production.

Based on technology established so far, two types of synthetic seeds are known: *desiccated* and *hydrated*. The desiccated synthetic seeds are produced from somatic embryos either naked or encapsulated in polyoxyethylene glycol (Polyox[r]) followed by their desiccation. Desiccation can be achieved either slowly over a period of one or two weeks sequentially using chambers of decreasing relative humidity, or rapidly by unsealing the petri dishes and leaving them on the bench overnight to dry. Such types of synseeds are produced only in plant species whose somatic embryos are desiccation-tolerant. On the contrary, hydrated synthetic seeds are produced in those plant species where the somatic embryos are recalcitrant and sensitive to desiccation. Hydrated synthetic seeds are produced by encapsulating the somatic embryos in hydrogel capsules.

The production of synthetic seeds for the first time by Kitto and Janick[8] involved encapsulation of carrot somatic embryos followed by their desiccation. Of the various compounds tested for encapsulation of celery embryos, Kitto and Janick[8–10] selected polyoxyethylene which is readily soluble in water and dries to form a thin film, does not support the growth of micro-organisms and is non-toxic to the embryo. Janick *et al.*[3] have reported that desiccated artificial seeds were produced by coating a mixture of carrot somatic embryos and callus in polyoxyethylene glycol. The coating mixture was allowed to dry for several hours on a Teflon surface in a sterile hood. The dried mixture was then placed on a culture medium, allowed to rehydrate, and then scored for embryo survival.

In 1984 Redenbaugh *et al.*[11] developed a technique for hydrogel encapsulation of individual somatic embryos of alfalfa. Since then encapsulation in hydrogel remains to be the most studied method of artificial seed production[4,7]. A number of substances like potassium alginate, sodium alginate, carrageenan, agar, gelrite, sodium pectate, etc. have been tested as hydrogels but sodium alginate gel is the most popular[5]. Hydrated artificial seeds consist of somatic embryos individually encapsulated in a hydrogel (Figure 1). To produce hydrated synthetic seeds, 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. Hardening of the calcium alginate is modulated with the concentrations of sodium alginate and calcium chloride as well as the duration of complexing. Usually 2% sodium alginate gel with a complexing solution containing 100 mM Ca^{2+} is used and is found to be satisfactory[5,7,12]. However, Molle *et al.*[13] found that for the production of synthetic seeds of carrot, 1% sodium alginate solution, 50 mM Ca^{2+} and 20–30 min time period were satisfactory 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. As a result, the embryos are positioned in the centre of the beads for better protection.

For the past several years other unipolar structures such as apical shoot tips and axillary shoot buds as well as apolar protocorms or protocorm-like bodies and even undifferentiated embryogenic calli are also being employed in synthetic seed production (Table 1). The technology of hydrogel encapsulation is also favoured for synthetic seed production from these micropropagules. For production of synthetic seeds from apical shoot tips and axillary shoot buds, these organs are usually first treated with auxins for root induction and then their microcuttings (approximately 4 or 5 mm in length) are

Figure 1. Somatic embryos of mango (*Mangifera indica* L.) encapsulated in calcium alginate capsule (embryos are approximately 3–5 mm long).

Table 1. List of plant species in which encapsulation technology has been applied to produce synthetic seeds

Plant	Propagule used for encapsulation	Reference
Actinidia deliciosa (Kiwifruit)	SBs	29
Arachis hypogaea (Groundnut)	SEs	21
Asparagus cooperi	SEs	20
Betula pendula (Birch)	SBs	29
Brassica campestris (Mustard)	SBs	56
Camellia japonica L.	SEs	57
Crataegus oxyacantha (Hawthorn)	SBs	29
Cymbidium giganteum (Orchid)	PLBs	30
Daucus carota (Carrot)	SEs	8–10
Dendrobium wardianum (Orchid)	PLBs	31
Eleusine coracana Gaertn. (Finger millet)	SEs	58
Eucalyptus citriodora (Eucalyptus)	SEs	59
Geodorum densiflorum (Lam) Schltr. (Orchid)	PLBs	32
Malus pumila Mill. (Apple rootstock M.26)	SBs	1, 28, 29
Mangifera indica L. (Mango cv. Amrapali)	SEs	12, 27
Medicago sativa (Alfalfa)	SEs	11
Morus indica (Mulberry)	SBs	60
Musa (Banana cv. Basrai)	SBs	15
Pelargonium × *domesticum* (Regal geranium)	SEs	61
Pelargonium × *hortorum* (Zonal geranium)	SEs	61
Phaius tankervillae (Orchid)	PLBs	33
Picea abies (Norway spruce)	SEs	62
Picea glauca (White spruce)	SEs	63
Picea glauca Engelmannii (Interior spruce)	SEs	64
Picea mariana (Black spruce)	SEs	64
Pinus taeda	SEs	62
Pistacia vera L. (Pistachio)	SEs & EMs	22
Psidium guajava (Guava)	SEs	65
Rubus idaeus L. (Raspberry)	SBs	29
Rubus (Blackberry cv. Jumbo, Veten)	SBs	29
Santalum album (Sandalwood)	SEs	14, 24, 26
Solanum melongena (Eggplant)	SEs	66
Spathoglottis plicata (Orchid)	PLBs	34
Syringa vulgaris L. (Lilac)	ABs	36
Vitis vinifera (Grape)	SEs	2
Zingiber officinale Rosc. (Ginger)	SBs	67

SEs, somatic embryos, SBs, shoot buds; ABs, axillary buds; EMs, embryogenic masses; PLBs, protocorm-like bodies.

encapsulated in sodium alginate gel following the method described by Redenbaugh *et al.*[11] for alfalfa somatic embryos. However, mulberry[14] and banana[15] plantlets were obtained from alginate-encapsulated shoot buds without any specific root induction treatment. To avoid bacterial contamination Ganapathi *et al.*[15] added an antibiotic mixture (0.25 mg/l) containing rifampicin (60 mg), cefatoxime (250 mg) and tetracycline-HCl (25 mg) dissolved in 5 ml dimethyl sulphoxide to the gel matrix. Activated charcoal (0.1%) was also added to the matrix to absorb the polyphenol exudates of the encapsulated shoots of banana[15].

Achievements and prospects

Somatic embryos

Although various micropropagules have been considered for synthetic seed production, the somatic embryos have been largely favoured (Table 1) as these structures possess the radicle and plumule that are able to develop into root and shoot in one step, usually without any specific treatment. The advantages of preparing synthetic seeds from somatic embryos have been discussed by Redenbaugh[16]. The use of somatic embryos as artificial seeds is becoming more feasible as the advances in tissue culture technology define the conditions for induction and development of somatic embryos in an increasing number of plant species[17]. Various types of artificial seeds have been prepared using somatic embryos which have been either dried[18,19] or maintained fully hydrated[6,12,20–22], these may or may not be encapsulated[9,10,23,24]. However, if the somatic embryo is dried to moisture content of approximately 10%, as in a number of true seeds, the propagation system has the additional advantage of serving as a germplasm storage system, which maintains the propagule in a quiescent state for extended periods of time. Dried somatic embryos would also provide a more efficient use of space and labour in a commercial production system and storage for planting in the future. Attempts have been made to desiccate somatic embryos with or without encapsulation to exploit this potential, but success has been relatively limited except for *Medicago sativa*[19].

In alfalfa (*M. sativa*) desiccation-tolerance of somatic embryos was induced by exogenous application of abscisic acid (ABA) by Senaratna, Mckersie and Bowley[19]. Subsequently, the embryos were dried to 10–15% moisture and stored for at least 3 weeks in the dry state. Under appropriate treatment conditions, 65% of these somatic embryos survived and germinated in a manner analogous to a true seed. Desiccation-tolerance has also been induced in alfalfa somatic embryos by exposure to sub-lethal levels of low temperature, water, nutrient or heat stress. However, these pretreatments had deleterious effects on embryo maturation and plantlet vigour[19].

Onishi, Sakamoto and Hirosawa[25] have demonstrated a protocol for the production of synthetic seeds involving automation at the production and encapsulation stages. These authors have emphasized that high and uniform conversion of synthetic seeds under a practical sowing situation, such as, nursery bed in a greenhouse or in the field, is an essential requirement for their use in clonal propagation of plants. They found that conversion of celery and carrot embryos produced in bioreactors, could be raised to 53–80% from 0% by three sequential treatments: (i) Culturing the embryos for 7 days in a medium of high osmolarity (with 10% manni-

tol) under 16 h photoperiod with 300 lux of illumination for promoting embryo development. This treatment increased the size of embryos from 1–3 mm to 8 mm and their chlorophyll content. (ii) Dehydration of embryos to reduce their water content from 95–99% to 80–90% by keeping them for 7 days on 2–7 layers of filter paper under a 16 h photoperiod of 14 μE m^{-2} s^{-1} irradiance. (iii) Post-dehydration culture on SH medium containing 2% sorbitol, 0.01 mg/l BAP and 0.01 mg/l GA_3, in air enriched with 2% CO_2 under a 16 h photoperiod at 20°C for 14 days to acquire autotrophic nature and reserve food. The bead quality was also modified by impregnating them with 3% sucrose, by coating the microcapsules with a fungicidal mixture comprising 8% Elvax 4260 and beeswax, and 0.1% Topsin M (ref. 25). To facilitate the emergence of shoot and root meristems during embryo germination, Onishi, Sakamoto and Hirosawa[25] have made the gel capsule self-breaking under humid conditions. It involved rinsing the beads thoroughly with running tap water, followed by immersion in a 200 mM solution of KNO_3 for 60 min and, desalting them by rinsing in running tap water for 40 min. Such synthetic seeds showed 50% conversion in two weeks after sowing in a greenhouse.

In tree species like *Santalum album*[24,26], *Pistacia vera*[22] and *Mangifera indica*[12,27] also the somatic embryos have been encapsulated to produce synthetic seeds. However, further research is needed to optimize protocols for production of viable synthetic seeds that could be stored for longer periods and could be commercially viable.

Axillary shoot buds and apical shoot tips

In many plant species (Table 1) the unipolar axillary shoot buds and/or apical shoot tips which do not have root meristem, have also been encapsulated to produce synthetic seeds. Since these structures do not have root meristems they should be induced to regenerate roots before encapsulation. Different authors[14,15] have described how encapsulated buds of banana and mulberry converted into plantlets without specific root induction treatments. In different reports Piccioni[28] and Capuano, Piccioni and Standardi[1] have described conversion of shoot buds of apple clonal rootstock M.26 encapsulated after an appropriate root induction treatment with IBA (24.6 μM) for 3–6 days. Capuano and coworkers[1] have found different conversion behaviour of the synthetic seeds made of axillary and apical microcuttings. They have reported that conversion of the synthetic seeds obtained with *axillary microcuttings* of M.26 apple rootstock always occurred at a very low rate (only 25%) following 6 days of root primordial initiation (RPI) culture and cold storage. In contrast, *apical microcuttings* reached 85% conversion with a 24.6 μM IBA treatment and 3 days of RPI culture without cold storage. These results confirm the suitability of such explants towards encapsulation and synthetic seed production. Besides, the results encourage the use of encapsulated unipolar explants, such as micropropagated buds for the synthetic seed technology. This kind of capsule could be useful in exchange of sterile material between laboratories due to small size and relative ease in handling these structures, or in germplasm conservation with proper preservation techniques[29], or even in plant propagation and nurseries, if the development of the plant could be properly directed towards proliferation, rooting, elongation, etc.[1,28,29].

Embryogenic masses

Stable and regenerative embryogenic masses make an attractive tool for the production of clonal plants and for studies of genetic transformation. However, long-term maintenance of embryogenic masses in culture tubes or mechanically stirred bio-reactors requires frequent transfer of tissue to fresh media which is both labour-intensive and costly. To cope up with these difficulties, the embryogenic masses of *Pistacia vera*[22] have been encapsulated in sodium alginate gel using the method of Redenbaugh *et al.*[11] and stored at 4°C after treatment with BAP. Onay, Jeffree and Yeoman[22] have reported that the encapsulated embryogenic masses recovered their original proliferative capacity after two months storage following two subcultures. Nevertheless, it remains to be established whether the storage period can be extended further, and also if the efficiency of embryogenic masses for production of somatic embryos declines during the long-term storage.

Protocorms or protocorm-like bodies

In orchids such as *Cymbidium giganteum*[30], *Dendrobium wardianum*[31], *Geodorum densiflorum*[32], *Phaius tonkervillae*[33] and *Spathoglottis plicata*[34] synthetic seeds have been produced by encapsulating the protocorm or protocorm-like bodies (PLBs) in sodium alginate gel. Corrie and Tandon[30] have reported that the encapsulated protocorms of *C. giganteum* gave rise to healthy plantlets upon transferring either to nutrient medium or directly to sterile sand and soil. They found that conversion frequency was high in both *in vitro* (100%) and *in vivo* (88% in sand, 64% in sand and soil mixture) conditions. These techniques have made it possible to transplant the aseptically grown protocorms directly in the soil, cutting down the cost of raising *in vitro* plantlets and their subsequent acclimatization[30].

Use of synthetic seeds appears to be particularly promising. The encapsulation, storage and re-growth of

homogeneous material allow the possibility of automated mass production of elite plant species[35,36]. There are several potential uses of synthetic seeds of those crop plants that are vegetatively propagated and have long juvenile periods, e.g. citrus, grapes, mango, etc. The planting efficiency of such crops could theoretically be increased by the use of synthetic seeds instead of cuttings. Synthetic seeds have been found highly advantageous for germplasm conservation in grape and other similar crops[2].

Limitations

Although results of intensive researches in the field of synthetic seed technology seem promising for propagating a number of plant species (Table 1), practical implementation of the technology is constrained due to the following main reasons:

- Limited production of viable micropropagules useful in synthetic seed production.
- Anomalous and asynchronous development of somatic embryos.
- Improper maturation of the somatic embryos that makes them inefficient for germination and conversion into normal plants.
- Lack of dormancy and stress tolerance in somatic embryos that limit the storage of synthetic seeds.
- Poor conversion of even apparently normally matured somatic embryos and other micropropagules into plantlets that limit the value of the synthetic seeds and ultimately the technology itself.

Development of artificial seeds requires sufficient control of somatic embryogeny from the explants to embryo production, embryo development and their maturation as well. The mature somatic embryos must be capable of germinating out of the capsule or coating to form vigorous normal plants. A number of researchers have tried to improve the quality[37–40] and quantity[27,41–43] of somatic embryos via modification of culture conditions, such as, medium composition, growth regulators (types and concentrations), physical state of the medium, as well as incubation conditions like temperature, illumination, etc.

Although large quantities of somatic embryos can be rapidly produced in many plant species, normal plants are difficult to obtain due to their improper or asynchronous maturation. Hence, maturation of somatic embryos, which eventually controls germination and conversion rate, is one of the major bottlenecks for synthetic seed production. While studying the effects of different types of osmotica on maturation of somatic embryos of spruce, Attree and Fowke[39] and Fowke and Attree[44] have described that inclusion of high levels of sucrose (i.e. permeating osmotica) in the standard medium containing ABA (which is associated with water stress), prevents maturation while inclusion of PEG (non-permeating osmotica) with ABA dramatically improves the frequency and synchrony of the somatic embryo maturation. Biochemical analysis of these somatic embryos showed a striking increase in storage lipids and proteins compared to the embryos matured without PEG.

For commercial applications, somatic embryos must germinate rapidly and should be able to develop into plants at least at rates and frequencies more or less similar if not superior to true seeds. To achieve conversion of somatic embryos into plantlets and to overcome deleterious effects of recurrent somatic embryogenesis as well as anomalous development of somatic embryos on their conversion, it is necessary to provide optimum nutritive and environmental conditions[27,45]. Maltose has been found valuable for improving alfalfa somatic embryo conversion[7]. From a synthetic seed perspective, addition of sucrose in the medium is necessary for viability of somatic embryos, their subsequent development, maturation and germination in many plant species[17,27,38,44].

In an *in vitro* culture system the somatic embryos show great diversity in their morphology and accordingly in their response which greatly limits the use of synthetic seed technology. Lee and Soh[46] have indicated that continuous ABA treatment increases the formation of somatic embryos with anomalous cotyledons, while in some instances ABA has been found to promote the normal development of both somatic and zygotic embryos *in vitro*[47,48]. Cytokinin treatment also increases the number of somatic embryos with multiple cotyledons[49]. It is suspected that the morphological development of somatic embryos is regulated by endogenous hormones[50]. Liu, Xu and Chua[51] have described the effect of anti-auxins on polar auxin transport which controls embryo development. For initiation of the two cotyledons, a polar auxin transport in the embryo is needed for a short period during the globular stage and developmental abnormalities occur due to cell divisions in the meristematic areas prior to differentiation of the shoot apex and cotyledons[50,51]. The developmental anomalies, however, are not intrinsic to somatic embryos, because immature zygotic embryos can also exhibit similar irregularities when removed from the seed and allowed to develop *in vitro*[52]. Choi *et al.*[50] have suggested that unbalanced endogenous hormone distribution by exogenous hormone treatment may result in the abnormal somatic embryos.

In many plant species the somatic embryos have been found to be sensitive to desiccation. Desiccation damages the somatic embryos and inhibits their germination and conversion into plants in desiccation-sensitive plant species[5,6]. Nevertheless, desiccation and subsequent rehydration have been found useful in inducing a high

frequency conversion of somatic embryos into plantlets in some species[18,53]. Gradual drying of alfalfa somatic embryos with progressive and linear loss of water gave better response and improved the quality of embryos in comparison to uncontrolled drying[7]. Similarly, desiccation improved the germination frequency in soybean[54] also. Senaratna *et al.*[55] have reported that desiccation-tolerance can be induced in somatic embryos of alfalfa by external stimuli such as ABA, exposure to cold, heat, water and osmotic stress at sub-lethal levels or increasing the sucrose content in the medium. Attree *et al.*[38] and Fowke and Attree[44] have reported that somatic embryos of spruce matured in the presence of PEG and ABA were very tolerant to low moisture levels. According to them, such somatic embryos had less than 50% moisture content which was further reduced to less than 10% following desiccation. These embryos were stored at –20°C for a year and thereafter successfully germinated following imbibition with no loss in viability.

The coating material may also limit success of the synthetic seed technology, and at present none of the embryo encapsulation methods described earlier is completely satisfactory. The hydrated capsules are more difficult to store because of the requirement of embryo respiration[6]. A second problem is that capsules dry out quickly unless kept in a humid environment or coated with a hydrophobic membrane[23]. Calcium alginate capsules are also difficult to handle because they are very wet and tend to stick together slightly. In addition, calcium alginate capsules lose water rapidly and dry down to a hard pellet within a few hours when exposed to the ambient atmosphere. These problems can be offset by coating the capsules with Elvax 4260 (ethylene vinyl acetate acrylic acid terpolymer, Du Pont, USA)[7]. Redenbaugh, Fujii and Slade[5] have reported that the limitations caused by coating materials can be overcome by selecting appropriate coating material for encapsulation. According to them, the coating material should be non-damaging to the embryo, mild enough to protect the embryo and allow germination and be sufficiently durable for rough handling during manufacture, storage, transportation and planting.

The concentration of the coating material is also an important limiting factor for the synthetic seed technology. The coat must contain nutrients, growth regulator(s) and other components necessary for germination and conversion and it should be transplantable using the existing farm machinery. Though many coating materials have been tried for encapsulation of somatic embryos, sodium alginate obtained from brown algae is considered the best and is being popularly used at present. Alginate has been chosen for ease of capsule formation as well as for its low toxicity to the embryo. The rigidity of the gel beads protects the fragile embryo during handling. According to Redenbaugh *et al.*[23], the capsule gel can potentially serve as a reservoir for nutrients (like an artificial endosperm) that may aid the survival and speed up the growth of the embryo.

Conclusions

Despite considerable research input into artificial seed production during the last fifteen years, several major problems remain with regard to its commercialization. The first 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. Additional factors responsible for poor germination of synthetic seeds are the lack of supply of nutrients and oxygen, microbial invasion and mechanical damage of somatic embryos. In fact, conversion is the most important aspect of the synseed technology, and still remains one of the factors limiting commercial application of this technology. Until recently, most reports on somatic embryogenesis focused only on the production of embryos and recovery of a few plants. Among tree species, regeneration of viable plantlets from somatic embryos is a frequently encountered problem. The bottleneck may occur at any of a number of stages including maturation, germination, shoot apex elongation, rooting of shoots or acclimatization. While treatments to overcome these bottlenecks vary with the plant species, one general approach can be to simulate the conditions experienced by zygotic embryos in seeds prior to germination. The desiccation process, which damages the embryo, and other problems associated with desiccated artificial seeds need resolution. Occurrence of high levels of somaclonal variations in tissue culture is another aspect to be considered seriously while recommending the use of artificial seeds for clonal propagation.

One of the future usage of synthetic seeds would be in germplasm conservation through cryopreservation. Either hydrated calcium alginate-based or desiccated polyoxyethylene glycol-based artificial seeds might be used, but it is likely that some degree of drying before cryopreservation would be beneficial.

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.

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