Meristem	A mass of undifferentiated cells present in the shoot buds and the apical root at the origin of the formation and growth of the shoots and the roots.
Mutant	An organism in which one or more character(s) has been changed at the gene level by physical or chemical treatment.
Particle bombardment	A technique to introduce genes into cells by gun bombardment using gold or tungsten microparticles, coated with the required DNA.
Polarity	In a cell, the establishment of two poles characterized by the segregation at each pole of different components of the cell.
Promoter	In chromosomes, a noncoding sequence of nucleotides which controls the expression of a gene.
Somatic	The somatic cells constitute the body of the plants or animals. They generally contains two sets of chromosomes each coming from one of the two parents.

See also: **Production Systems and Agronomy**: Nursery Stock and Houseplant Production; Plantation Crops and Plantations. **Seed Development**: Artificial Seeds; Embryogenesis. **Tissue Culture and Plant Breeding**: Clonal Propagation, Forest Trees; Regeneration of Fruit and Ornamental Trees via Cell and Tissue Culture. **Tissue Culture**: Artificial Seeds; Clonal Propagation, *In Vitro*; General Principles; Micropropagation; Organogenesis.

Further Reading

Dodeman VL, Ducreux G, and Kreis M (1997) Zygotic embryogenesis versus somatic embryogenesis. *Journal of Experimental Botany* 48: 1493–1509.

Dudits D, Gyorgyey J, Bogre L, and Bako L (1995) Molecular biology of somatic embryogenesis. In: Thorpe TA (ed.) In vitro *Embryogenesis in Plants*, pp. 267–308. Dordrecht: Kluwer Academic.

Mordhorst A, Toonen MAJ, and de Vries S (1997) Plant embryogenesis. *Critical Reviews in Plant Sciences* 16: 535–576.

Schmidt EdDL, de Jong AJ, and de Vries S (1994) Signal molecules involved in plant embryogenesis. *Plant Molecular Biology* 26: 1305–1313.

Sorvari S, Toldi O, Ahanen K, *et al.* (1997) Using polysaccharides and galactomannans as gelling agents in capsule formation of artificial seeds. *Journal of the American Society for Horticultural Science* 122: 878–883.

Zimmerman JL (1993) Somatic embryogenesis: a model for early development in higher plants. *Plant Cell* 5: 1411–1423.

Artificial Seeds

S Pond and S Cameron, Canadian Forestry Service, Fredericton, NB, Canada

Definition

Strictly speaking, the definition of an artificial seed (also called a synthetic seed or synseed, seed analog, or manufactured seed) is a somatic embryo inside a coating; it may be sown in the same manner as a conventional seed. Somatic (nonzygotic) embryos are bipolar (having both root and shoot meristems), arise from somatic (nonsexual) cells, and are genetically identical. Zygotic embryos are a result of sexual reproduction (fusion of gametes) and are not genetically identical making them less useful for the production of artificial seeds. The somatic embryo may be hydrated or desiccated; the coating may be water impermeable or water soluble and may also enclose nutrients (artificial endosperm) and other additives deemed necessary (e.g., mycorrhizal fungi, fungicides, and/or bacteriocides). The term "artificial seed" may also refer to unencapsulated (naked) somatic embryos (either hydrated or desiccated). Due to the low success rate and/or high cost of somatic embryo production in some species, buds, shoots, bulbs, or other meristematic tissues that can produce a whole plant may also be encapsulated. These are also considered to be artificial seeds. The type of artificial seed produced and its economic feasibility vary greatly among species, depending on the ease and cost of natural reproduction (i.e., by seed or by vegetative propagation) and the state of current technology in somatic embryogenesis for that species. The decision to use artificial seed technology depends on the plant species, the need for improvements in production efficiency, or other justification for the development and production costs associated with artificial seed.

Role of Natural Seed and Artificial Seed in Plant Propagation

The seed is the delivery system in sexually reproducing plants that passes on genetic material from one generation to the next. A natural seed develops from a fertilized ovule and consists of a zygotic embryo with a nutritive supply surrounded by a protective coat. The seed structures surrounding the embryo protect it during storage and sowing, and nourish it during germination. They also regulate gas exchange

(which influences embryo respiration), and embryo development, and prevent precocious germination. The majority of seeds from temperate species tolerate desiccation – i.e., they can survive without water for long periods of time – and in this anhydrous state can survive extremely adverse conditions for many years. They can also be kept in frozen storage or in liquid nitrogen for long-term germplasm storage. These dried orthodox seeds can be mechanically planted directly in the field in a cost-efficient manner. Automated machinery is readily available to handle conventional seed. These attributes make seeds the most feasible and cost-efficient means of storing and propagating plants.

However, there are drawbacks to the use of natural seeds as the sole units of propagation. Many plants are poor seed producers and require supplemental methods of propagation to decrease production costs and supply sufficient quantities of plant material to meet demands. In other cases, the plants do not breed true. Sexual reproduction recombines specific genes, making it impossible to preserve unique gene combinations. Inbred strains also often result in decreased vigor and lower seed productivity. In order to produce an exact copy or clone of the original plant, these species or varieties must be vegetatively propagated. This has been done very successfully in many plant species. Many tropical species have recalcitrant seeds (i.e., they are sensitive to chilling or desiccation and cannot be stored) and thus require alternative methods to store and propagate the germplasm. Diseases are intrinsic in seeds of some species, so other means of propagation are needed to produce disease-free plants. In some species with long reproductive cycles (gymnosperms being a prime example) where the time from germination to first flowering is measured in years, production of natural seed from a desired cross is a very slow process.

Vegetative reproduction through tissue culture can circumvent the above problems. Large numbers of identical clones can be produced in a short period of time and a cost-effective manner; sterile or unstable genotypes can be multiplied efficiently; and tissue can be cryopreserved to permit long-term storage of germplasm. Tissue culture is used to produce disease-free plants, and genetically engineered plants are initially multiplied using *in vitro* methods.

There are a number of ways to vegetatively propagate plants through tissue culture, such as organogenesis and meristem culture. However, the most promising method for the development of encapsulable units suitable for artificial seed is somatic embryogenesis (SE). Somatic embryogenesis mimics the zygotic embryogenesis that occurs in a natural seed. Superficially similar stages are produced in most species in somatic and zygotic embryogenesis, each resulting in an embryo (either somatic or zygotic, respectively) with shoot and root apices ready to germinate. Somatic embryogenesis has the potential to economically produce unlimited numbers of identical somatic embryos that can be germinated into crops of identical plants. However, these embryos are naked. The tissue culture nutrient medium and not the mother plant provides nutrition. Availability of oxygen and other gases is regulated, often poorly, by the culture environment and not by the protective layers of the seed coat. As a result, developmental problems may occur during maturation of the somatic embryos because of nonoptimal culture methods. These problems must be addressed before embryo production and artificial seed production can be automated.

Naked somatic embryos can be used as artificial seeds, but they are not quiescent and they lack the nutritive and protective layers present in a natural seed. The embryo epidermis is unlikely to have protective waxy layers. Therefore, embryos are germinated under laboratory conditions (*in vitro*) until they become photoautotrophic, after which they are gradually hardened off. They are then transplanted to a greenhouse where they are further acclimatized, at which point they can be treated as normal seedlings. This process is labor intensive and costly. If these naked embryos were encapsulated with nutritive material and a protective coating, they would hypothetically be protected during handling and germination, and would receive nutrients and/or other factors to enhance germination. Thus, a coating is seen as a way to germinate artificial seed in less demanding conditions, and reduce handling costs. Desiccation of the embryos prior to encapsulation can induce quiescence, allowing them to be stored without loss of vigor. The ultimate goal in the production of artificial seeds is to produce a coated propagule as cost efficiently as natural seed. Ideally, the propagule could be stored for long periods of time without germinating or losing vigor. It would germinate and produce a whole viable plant when transferred to conditions conducive to germination; also, it would be strong enough to withstand the stress of mechanical seeding equipment. However, many technical and biological barriers still prevent attainment of these goals.

History

In 1902, J. Gottleib Haberlandt predicted that artificial embryos would be successfully cultivated from vegetative cells. In 1958, Stewart and Reinhart

were the first researchers generally acknowledged to have proved this prediction by independently discovering SE in carrot (*Daucus carota*) plants. Earlier reports by Levine in 1947 and Curtis and Nichol in 1948 describe structures, in carrot and orchid (Orchidaceae) respectively, that may have been somatic embryos.

At a conference in 1977, Toshio Murashige first publicly addressed the concept of artificial seeds and, since then, different approaches have been actively pursued to make it a viable technology, with varying levels of success. In the mid-1970s, Keith Walker's group at Monsanto worked on alfalfa (*Medicago sativa*, lucerne), soybean (*Glycine max*), and other vegetable crops while Robert Lawrence's group at Union Carbide began working on forest trees, celery (*Apium graveolens*), and lettuce (*Lactuca sativa*) using fluid drilling and polyoxyethylene for seed tapes or sheets. In the 1980s, Redenbaugh and coworkers developed sodium alginate hydrogels for single embryo artificial seeds and were able to produce hydrated artificial seeds of alfalfa and celery, although they had very low conversion rates (7–10%, respectively). Work on encapsulation of hydrated embryos continues today. Research is ongoing at Weyerhauser Ltd as part of their integrated Miniplug® system on a sophisticated botanic seed analog for conifer somatic embryos which incorporates a perfluorocarbon-oxygenated nutrient medium separated from the embryo by a porous membrane, and covered with a rigid waterproof seed coat with a weak spot for root emergence.

In the 1980s, work also started on desiccation of embryos for use as artificial seed. In 1985, Gray and Conger reported plant recovery (4%) from desiccated quiescent orchard grass (*Dactylis glomerata*) somatic embryos. This was repeated with grape (*Vitis vinifera*) somatic embryos in 1987 (equilibrated to 13% water content, stored for 21 days with 34% germinating and converting after imbibition). McKersie's group in 1989 kept alfalfa somatic embryos desiccated for 1 year with a 60% survival rate after imbibition. Compton and coworkers were able to germinate plants from dried corn and soybean embryos in 1992 at about a 60% success rate. Attree and colleagues in 1991 and 1992 recovered viable plants from 81% of interior spruce embryos after dry storage for 14 days (using embryos matured in a medium with polyethylene glycol as the osmoticant) and, in 1999, patented his desiccation method and an encapsulation method which uses a melted nonhydrated water-soluble compound (polyethylene glycol) to coat the desiccated embryos and yield a hardened capsule when solidified. When planted, the capsule gradually dissolves after exposure to water.

The journal and patent literature is currently filled with accounts of attempts to commercialize artificial seed technology for fruits and plantation crops, vegetable crops, ornamental crops, spice, medicinal/aromatic plants, and trees. There have been many modest successes published in the development of desiccated artificial seed but, in most cases, too many biological and/or technological problems remain to use the technology on a commercial basis.

Feasibility of Producing Artificial Seeds

As discussed previously, there are a number of possible artificial seed systems. The type of artificial seed produced, based on its economic feasibility, will vary greatly among species, depending on the ease and cost of natural reproduction (i.e., by seed or by vegetative propagation), the state of current SE technology for that species, the need for improvements in production efficiency, or other justification for the development and production costs associated with artificial seed and the end use of the artificial seed (e.g., storage, greenhouse germination, field germination). As stated by Murashige and Street in the 1970s, and as has been proved consistently in the literature, the major stumbling block in establishing artificial seed production as a viable technology is a lack of understanding of the SE process and an inability to consistently produce high-quality propagules that can germinate in a soil environment with an acceptably high success rate.

The development of the ideal viable, quiescent, low-cost artificial seed was described as a 10-step process by Redenbaugh in 1987. Some steps generally apply to more than one species whereas other steps may be species-specific. The latter are inevitably the most demanding in terms of development, and are noted as such.

1. Selection of the crop based on technological and/or commercial potential (species-specific).
2. Establishment of an SE system (species-specific).
3. Optimization of the clonal production system (optimizing protocols to synchronize and maximize the development of normal mature embryos capable of conversion to normal plants) (species-specific).
4. Automation of embryo production.
5. Posttreatment of mature embryos to induce quiescence (which may involve a desiccation step).
6. Development of an encapsulation and coating system.
7. Optimization and automation of the encapsulation system.

8. Optimization of conversion requirements for greenhouse and field growth (watering, fertilizer, transplantation, etc.).
9. Identification and control of any pest and disease problems that may be unique to artificial seeds.
10. Determination of the economic feasibility of using the artificial seed delivery system for a specific crop compared with other propagation methods (cost–benefit analysis of encapsulation versus other options).

Methods of Producing Artificial Seeds

Hydrated Somatic Embryos

Naked embryos Naked, hydrated, artificial seeds have the lowest development cost of all the artificial seed systems using somatic embryos, but have the highest handling and germination costs (**Figure 1**). They require a well-established, cost-efficient SE system capable of routinely producing large numbers of high-quality somatic embryos showing good conversion percentages. Hydrated embryos are nonquiescent and must be germinated immediately after production or stored under moist conditions for only very short periods of time. They are fragile and, in most cases, require careful, individual handling that increases the cost per seed. They are germinated *in vitro* under well-controlled conditions and transferred manually through various hardening-off procedures before transplantation to the greenhouse.

This method may be applied to high-value ornamental crops where the high end-value of the crop justifies the high cost per plant. An example of a crop that uses this method of propagation is Colorado blue spruce (*Picea pungens*) in which only a small percentage of trees have good color. They are selected from seed-grown nursery stock at 3–7 years of age for color and form. The parent trees are insufficiently juvenile by then to be propagated by any means other than by grafting which results in a cost to the consumer of US$60.00 per tree. Propagating blue spruce trees using SE allows a portion of the SE tissue from each clone to be frozen while the remainder is used to produce somatic embryos. The embryos are germinated and planted, after which the seedlings are observed for several years for development of the desired blue color. The tissue from the clones that produce the best color can then be thawed and used for large-scale production of naked, hydrated somatic embryos at a lower cost than grafted trees.

Although costly, mass propagation using naked embryos has the benefit of circumventing the biological and technological limitations involved in coating and/or drying somatic embryos. Machinery has been developed to automate planting of *in vitro* germinated somatic embryos into sterile plugs for greenhouse rearing (Fast Plant® developed by Silvagen, Halifax, Nova Scotia and currently used in interior spruce) that decreases handling costs.

Encapsulated embryos Hydrated mature somatic embryos can be encapsulated in a hydrogel (**Table 1**). The majority of the published reports discuss this type of artificial seed (**Figure 2**). The coating should be nontoxic and offer protection from mechanical damage while providing water and nutrients to the embryo during storage and sowing. However, there are problems associated with the use of hydrogel capsules. In some species, embryo vitrification due to aberrant water relations is a problem. Gas exchange through the gel may inhibit growth, and nutrients can leach from the beads. Excessive gel firmness may inhibit germination. The gel provides an excellent nutrient medium for microorganism growth and,

Figure 1 Germination of hydrated, naked white spruce embryos on sorbarods: (A) at 3 days; (B) at 14 days. Reproduced with permission from Maruyama E, Kinoshita I, Ishii K, *et al* (1997) Alginate-encapsulated technology for the propogation of tropical trees: *Cedrela odorata* L., *Guazuma crinita* Mart., and *Jacaranda mimosaefolia* D. Don. *Silvae Genetica* 46: 17–23.

Table 1 Coating substances and additives for somatic embryos

Hydrogels[a]	*Additives*	*Dry coatings (or coatings applied hydrated and then dried)*
Sodium alginate	Fertilizer	Polyethylene glycol 1000–4000
Potassium alginate	Nutrient salts (N,P,K)	Polyethylene oxide homopolymers
Guar gum	Cytokinins	Acrylic copolymer containing carboxyl group
Agar	Humic acids	Potassium propenoate-propenamide copolymers
Agarose	α-Keto acids	Carboxyl methylized cold soluble swelling starch
Amylase	Gibberellic $acid_3$	Synthetic trioctachedral smectite
Pectin	Gibberellic $acid_{4/7}$	Synthetic sodium-magnesium lithium silicate
Dextran	Pesticides (ethazol, thiophantenethyl, fenaminosulf, ethazol, benomyl, chloroneb, captan, metalaxyl)	Starch + synthetic polymer of acrylamide
Gelatin	Biocontrolagents (Trichoderma harsianum, T. kininoii, Rhizobium)	Sodium acrylate
Starch	Sucrose	
Amylopectin	Activated charcoal	
Mannan		
Modified cellulose (e.g., carboxymethyl cellulose, polyacrylamide)		
Tragacanth gum		
Carrageenan with locust bean gum		
Polysaccharides and galactomannans + starch		
Sodium pectate		

[a] Singly or in combination

Figure 2 Alginate-encapsulated embryos of Norway spruce. Reprinted with permission from Gupta PK, Timmis R, Pullman G, *et al.* (1991) Development of an embryogenic system for automated propagation of forest trees. In: Vasil IK (ed.) *Scale-Up and Automation in Plant Propagation*, pp. 75–93. New York: Academic Press.

therefore, requires sterilization (which affects gel characteristics) and planting into a sterile environment or the addition of additives to control microbial growth. The gel capsules are also susceptible to dehydration and require specialized care during handling, sowing, and germination (e.g., at the pre-greenhouse transplant stage). Hydrated capsules can survive only short-term storage under cold, moist conditions (~1 month). The capsules are fragile and incompatible with most conventional seeding machines, requiring development of machinery engineered specifically for gel-encapsulated embryos. Singulation may be a handling problem because uncoated capsules tend to be sticky.

Attempts are being made to improve gel bead characteristics to address some of these problems. The hardness of the gel can be lowered using special alginate formulations, with or without the addition of substances that interfere with the hardening reaction. Adding inactive substances (inert granules, powders, air bubbles) may prevent the formation of a continuous gel matrix. Sakamoto and coworkers developed self-breaking beads that swell gradually after being sown in humid and low electrolytic conditions until they split and break open. Inert oxygen-carrying compounds such as perfluorocarbons and silicone have been added to the gel matrix to address the problem of oxygen availability. The gel matrix (or artificial endosperm) may contain additives (**Table 1**). Mamiya's group used sodium alginate with slow-release sucrose capsules to encapsulate asparagus (*Asparagus officinalis*) somatic embryos. The slow-release sucrose worked well, but as has been seen in other species, total covering of the embryo by the gel matrix interfered with normal germination of the embryo.

To protect the hydrated beads from desiccation, various coating strategies have been proposed. Some examples are: membrane-like coatings with wax and resin; polyorganosiloxanes; a thin membrane of alginate with a liquid center; a self-breaking, liquid-center capsule; and a dried water-soluble polymer coating (Elvax 4260). Tackiness can be minimized by rolling the capsules in a suitable powder (Tullanox).

A hydrated gel with or without additives can be used as an artificial endosperm and be surrounded by a rigid manufactured seed coat. The seed coat can be made of cellulosic material, glass, plastic, a cured

polymer resin, paraffin, wax, varnish, etc. usually with an orifice covered with a thin film layer to allow protrusion of the radicle during germination (as described in several published patents). These hydrated encapsulated artificial seeds are suitable for plants that have a high unit value (such as vegetable transplants), as they are relatively expensive to develop and have a high unit cost per seed.

Germinating embryos also may be planted using fluid drilling. Fluid drilling was originally developed as a crop establishment technique using a thickened gel matrix (in contrast to the solidified matrix used in encapsulated embryos). Germinated seeds are incorporated into a protective gel mixture that is then continuously extruded into the seedbed. The use of the gel with the addition of sucrose, fertilizers, plant growth hormones, pesticides, microorganisms, etc. results in greater and more uniform seedling emergence with earlier and greater yields, and allows bulk handling of many small plantlets. Gels for fluid drilling include: Laponite (magnesium silicate clay), Liqua-gel (potassium starch acrylamide), Terrasorb (starch or synthetic copolymer), Hydrozorb 30 (potassium acrylate), N-gel (various cellulose based materials – Natrosol). Natrosol has the highest oxygen diffusion rate.

Strict control of seedbed conditions is critical to allow seedlings to establish. This method has been applied to germinating carrot somatic embryos. However, very low conversion percentages were reported. There are still biological and technical problems to be overcome regarding normal development of embryos, interaction of embryos with the gel mixture (e.g., oxygen diffusion rate and level of nitrogen source), and definition of seedbed conditions for optimum germination. The method remains quite labor intensive as it requires controlled germination of the plants before they are fluid drilled.

Desiccated Somatic Embryos

Naked embryos Desiccation induces quiescence in somatic embryos, providing more handling flexibility in large-scale production systems. Dehydrated, naked, quiescent somatic embryos may be frozen for indeterminate germplasm storage or stored at room temperature for shorter periods of time. Desiccation allows somatic embryos of seasonal crops (e.g., vegetables for spring planting, trees for reforestation) to be produced year-round, stored, and distributed for germination as required. Desiccation has the added advantage of improving germination. Mild desiccation has been shown to cause a developmental switch from storage reserve synthesis to storage reserve catabolism, yielding more vigorous seedlings that exhibit more rapid and uniform development.

Unencapsulated desiccated embryos have the disadvantage of being brittle (especially the cotyledons if they are reflexed, or spread open), requiring extra care in their handling to prevent mechanical damage. Gentle handling and germination under controlled conditions in the laboratory or greenhouse are required, thus increasing the cost per seed. Overall, development costs are lower because no encapsulation is required. Not only must SE technology be capable of producing low-cost, high-quality somatic embryos, but the embryos must also be capable of withstanding desiccation and rehydration without suffering damage. Therefore, both optimized maturation and postmaturation protocols must be defined that increase storage product reserves in the embryos and increase desiccation tolerance. Currently this is the major stumbling block in the development of this technology in many species. Desiccation tolerance is the result of several mechanisms working together to limit water loss, protect cell

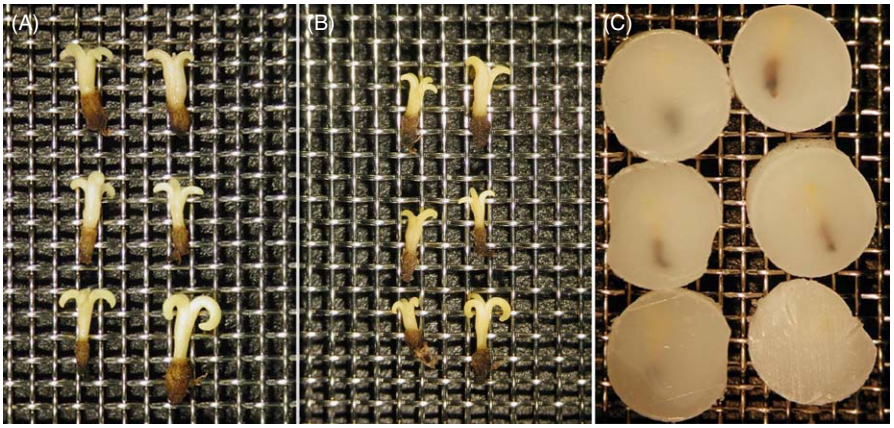

Figure 3 Nonaqueous encapsulation of white spruce. (A) Desiccated, cold-stored embryos; (B) embryos after rapid dehydration to $<10\%$ moisture content; (C) embryos in discs of polyethylene glycol 1000.

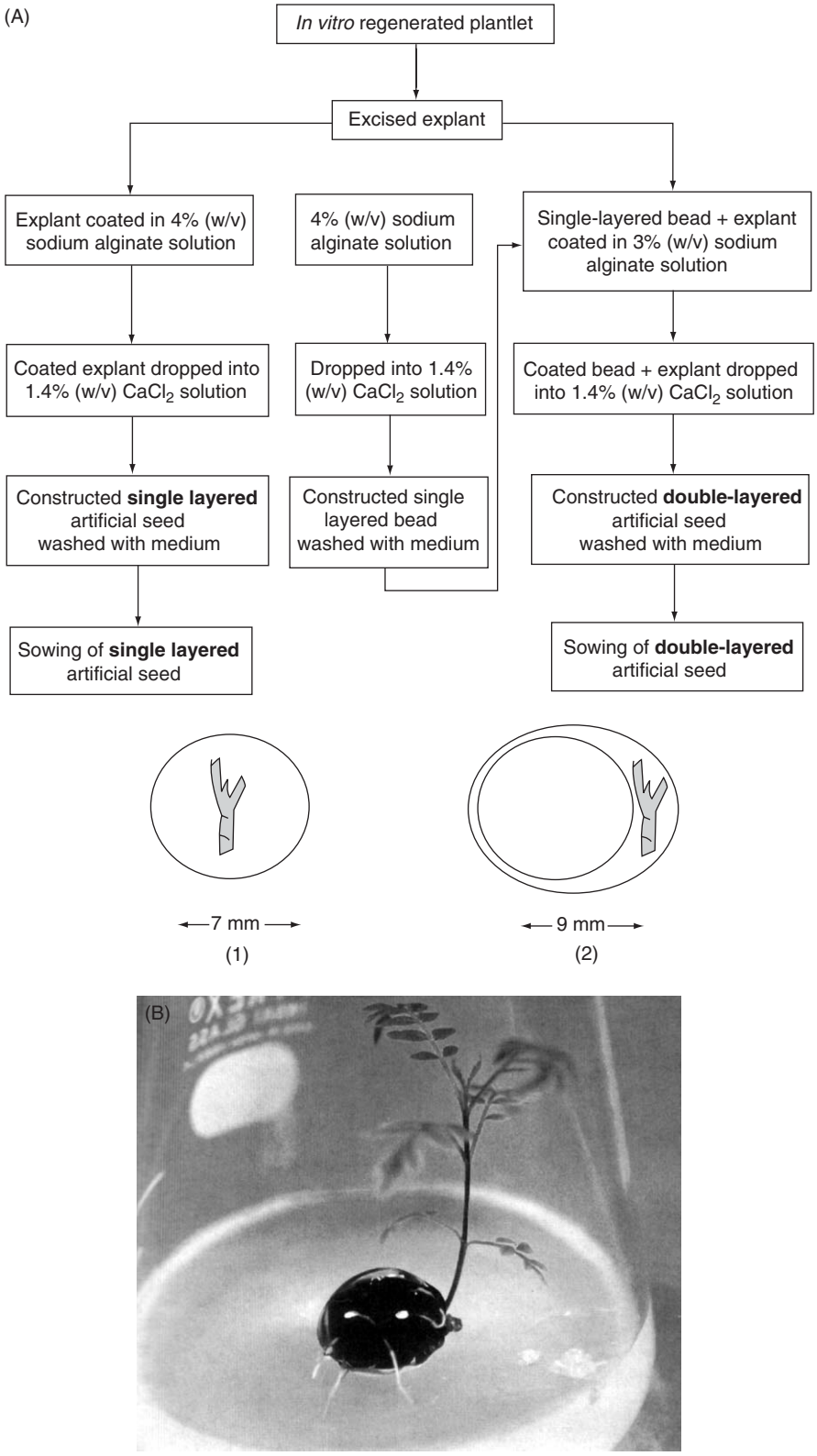

Figure 4 Double-layer encapsulation of meristems in alginate. (A) Schematic of the process; (B) germination of a *Jacaranda mimosaefolia* meristem from a double-layered bead containing charcoal. Reprinted with permission from Maruyama E, Kinoshita I, Ishii K, *et al.* (1997) Alginate-encapsulated technology for the propagation of tropical forest trees: *Cedrela odorata* L., *Guazuma crinita* Mart., and *Jacaranda mimosaefolia* D. Don. *Silvae Genetica* 46: 17–23.

membranes from damage during desiccation, and repair mechanisms that correct minor damage that has occurred during rehydration. Methods that increase the amount of time the embryos spend in the maturation process provide embryos with larger storage reserves and increased desiccation tolerance. Exposure to exogenous abscisic acid (ABA) and sublethal stress is important in promoting desiccation tolerance. Maturation media with high osmotic potential induced by either increased levels of permeating osmoticants (e.g., sucrose, mannitol), nonpermeating osmoticants (e.g., polyethylene glycol 4000) or higher gel strength media (to limit water availability), induce water stress, delay germination, increase storage reserve production, and increase desiccation tolerance. Other sublethal stresses (e.g., low temperature, nutrient deprivation) can have similar effects on desiccation tolerance. Properly pretreated embryos remain viable when they are rapidly desiccated to less than 15% moisture content.

Encapsulated embryos Coated desiccated embryos represent the ideal form of artificial seed. All the advantages of desiccated embryos are present, with the added advantage of encapsulation for handling. Encapsulated embryos can be tailored to the required size and shape necessary for use with conventional seed planting equipment. Desiccated somatic embryos destined for storage and/or field sowing must have abundant storage reserves and high conversion percentages (i.e., the percentage of embryos capable of converting into normal actively growing plants). Like their naked counterparts, they require as starting material quiescent somatic embryos capable of withstanding desiccation and rehydration and thus capable of surviving periods of cold, frozen, or room-temperature storage. The desiccated embryos are coated with a protective layer to protect them from mechanical damage during seed handling and may incorporate a nutritive layer to nourish and protect them from desiccation during the early stages of germination (**Figure 3**). The coating(s) must be nontoxic, nonaqueous (so they can be applied without rehydrating the embryos), and either meltable at a relatively low temperature (so the embryo does not suffer thermal damage during coating) or dry and attached to the embryos with an adhesive. The coating needs to dissolve readily in water after sowing to allow unimpeded germination of the embryo.

As with other methods, large numbers of normal mature somatic embryos must be generated in a cost-efficient manner to make nonhydrated artificial seeds competitive in price with natural seeds. As well, there should be some economic rationale for using artificial seed, such as the production of improved stock in conifers or in other species where the reproduction rate is slow, sporadic, or not true-to-type. Development costs are high in this type of artificial seed because rigorous protocols are needed to produce embryos able to withstand desiccation, as well as nonaqueous encapsulation media plus automated coating technology. Operational costs of actual seed production should be low once the development phase is past.

Table 2 Examples of artificial seeds in horticultural and plantation crops

Type of artificial seed	*Coating*
Hydrated naked somatic embryos	
Over 300 species form somatic embryos many of which could be used for artificial seed e.g., Colorado blue spruce	—
Hydrated encapsulated somatic embryos	
Alfalfa, asparagus, bamboo, *Brassica*, *Camellia japonica*, caraway, carrot, celery, cinnamon, coffee, *Coptis chinensis*, eggplant, geranium, ginger, horseradish, lettuce, lily, papaya, rice, sandalwood, tangerine, spruce (Norway, white, black)	Alginate
Dried, naked somatic embryos	
Alfalfa, carrot, grape, orchardgrass, spruce	—
Dried, encapsulated somatic embryos	
Brassica, carrot, papaya, interior spruce	Polyox, calcium alginate, sodium alginate with cellulose derivative A, Polyethylene glycol 1000
Other meristematic tissue, encapsulated	Alginate
1. Axillary buds Mulberry, kiwifruit, blackberry, raspberry, swamp cabbage	
2. Adventitious buds Vanilla, olive	
3. Shoot tips Banana, cardamon, apple, *Solanum* sp., oak, birch, tropical tree species	
4. Protocorms Orchid, *Cymbiidium giganteum, Dendrobium wardianum*	
5. Nodal segments *Dianthus, Solanum* sp.	

Other Meristematic Tissue

Axillary buds, shoot tips, and other meristematic tissue In cases where an SE system has not yet been developed due to the difficulty of producing embryogenic tissue, or problems with somatic embryo maturation, organogenically derived tissue can be used as the propagule. Propagules such as tissue culture-propagated axillary buds, adventitious shoots, shoot tips, protocorms, or hairy roots with shoots, are encapsulated in a manner similar to the hydrated somatic embryos, usually in a sodium alginate solution with nutrients, antibiotics, and often with activated charcoal (**Figure 4**). The propagules can then be planted on soil or other substrates such as cotton, filter paper, or soilrite. This method has been used successfully on a small scale in many horticultural and forestry crops (**Table 2**).

Conclusion

After 25 years of investigation, artificial seed technology remains a work in progress. Successful commercialization of any of the naked or coated embryo systems still requires substantially more knowledge of the basic mechanisms behind SE. Some problems to be overcome are: loss of embryogenic potential with age of the SE culture; asynchronous development and lack of mature embryo uniformity; precocious germination; structural anomalies; lack of desiccation tolerance; need for a sugar source for germination (heterotrophism); and low conversion percentages. In most species, these problems are caused by inappropriate cultural conditions and not factors intrinsic to SE. In the few species where substantial SE cultural advances have been achieved, researchers are well on their way to developing artificial seed.

Bulk production methods for economically producing tens or hundreds of thousands of propagules are rapidly evolving and being improved. The goal is a reliable, cost-effective SE system to synchronize the production of large numbers of high-quality somatic embryos showing high conversion percentages without encapsulation, and capable of surviving the stresses associated with encapsulation and sowing. The capability to enter a quiescent state upon desiccation and be stored in this dried state would be advantageous, as would the ability to be germinated in nonsterile conditions in the laboratory or greenhouse/field. Desiccation is expected to reduce handling costs after encapsulation, because the embryos can be handled like natural seed.

Aside from the costs to develop the encapsulable units for artificial seeds, additional investments are necessary to develop encapsulation methods and machinery for seed handling both during production and sowing. In the absence of front-end development costs, embryos will be fragile, incurring high handling expenses and the use of specialized greenhouses or laboratory space for germination and conditioning, thereby restricting use of the technology to only the highest-value crops due to the high unit cost. Nonetheless, for some crops (i.e., where SE systems are difficult to develop, or where synchronization, maturation, or germination problems persist), sowing naked propagules or encapsulation of hydrated tissue (e.g., shoots, buds, bulbs) may be the only alternative. After the initial expenditure, it is anticipated that artificial seeds can be produced quite cheaply by automating both embryo production and encapsulation.

Only after reliable, species-specific methods of bulk somatic embryo production tailored for artificial seed use have been devised can universal encapsulation and automation systems be developed. Although some progress has been made in demonstrating the feasibility of artificial seed and implementation has been successfully accomplished on a small scale, commercial use of artificial seed is still more a concept than a reality.

List of Technical Nomenclature

Conversion percentage	The percentage of somatic embryos that "convert" to growing plantlets with normal elongating hypocotyls and roots.
Encapsulable unit	An embryo, somatic embryo, bud, adventitious shoot, etc. that can be encapsulated, but which must have a high conversion percentage in its unencapsulated form and show a tolerance to the stresses associated with the encapsulation and sowing.
Heterotrophic	The type of plant that requires a source of carbohydrates as its carbon source.
In vitro	From the Latin meaning "in glass," referring to plant material that is cultured in aseptic conditions on artificial nutrient medium.
Photoautotrophic	The type of plant that uses CO_2 as its carbon source (by using photosynthesis) and does not require a source of carbohydrates (e.g., sugars) in the growing medium as a carbon source.
Propagule	A plant unit or structure (e.g., somatic embryo, protocorm, shoot tip) that will give rise to new plants.

Somatic embryo	An embryo produced from somatic cells or tissues and not as a result of fertilization of an egg cell.
Somatic embryogenesis	The process whereby somatic cells give rise to bipolar structures called somatic embryos.
Zygotic embryo	A bipolar structure produced as a result of fertilization of an egg cell.

See also: **Biodiversity and Conservation**: Seed Banks. **Postharvest Physiology**: Seed Storage. **Seed Development**: Seed Production. **Tissue Culture and Plant Breeding**: Clonal Propagation, Forest Trees; Regeneration of Fruit and Ornamental Trees via Cell and Tissue Culture. **Tissue Culture**: Somatic Embryogenesis.

Further Reading

Attree SM and Fowke LC (1999) *Maturation, Desiccation and Encapsulation of Gymnosperm Somatic Embryos*. United States Patent No. 5,985,667.

Cervelli R and Senaratna T (1995) Economic aspects of somatic embryogenesis. In: Aitken-Christie J, Kozai T, and Smith MAL (eds) *Automation and Environmental Control in Plant Tissue Culture*, pp. 29–64. Boston: Kluwer Academic Publishers.

Gray DJ, Compton ME, Harrell RC, and Cantliffe DJ (1995) Somatic embryogenesis and the technology of synthetic seed. In: Bajaj YPS (ed.) *Biotechnology in Agriculture and Forestry,* Vol. 30, *Somatic Embryogenesis and Synthetic Seed I*, pp. 126–151. Berlin: Springer-Verlag.

Gupta PK, Timmis R, Pullman G, *et al.* (1991) Development of an embryogenic system for automated propagation of forest trees. In: Vasil IK (ed.) *Scale-Up and Automation in Plant Propagation*, pp. 75–93. New York: Academic Press.

Hay IE and Charest PJ (1999) Somatic embryo germination and desiccation tolerance in conifers. In: Jain SM, Gupta PK, and Newton RJ (eds) *Somatic Embryogenesis in Woody Plants*, Vol. 4, pp. 61–96. Boston: Kluwer Academic Publishers.

Kitto SL, Pill WG, and Molloy DM (1995) Fluid drilling as a delivery system for somatic embryo-derived plantlets. In: Bajaj YPS (ed.) *Biotechnology in Agriculture and Forestry,* Vol. 30, *Somatic Embryogenesis and Synthetic Seed I*, pp. 181–192. Berlin: Springer-Verlag.

Maruyama E, Kinoshita I, Ishii K, *et al.* (1997) Alginate-encapsulated technology for the propagation of tropical forest trees: Cedrela odorata L., *Guazuma crinita* Mart., and *Jacaranda mimosaefolia* D. Don. *Silvae Genetica* 46: 17–23.

McKersie BD, van Acker S, and Lai F-M (1995) Role of maturation and desiccation of somatic embryos in the production of dry artificial seed. In: Bajaj YPS (ed.) *Biotechnology in Agriculture and Forestry,* Vol. 30, *Somatic Embryogenesis and Synthetic Seed I*, pp. 152–169. Berlin: Springer-Verlag.

Redenbaugh K (ed.) (1993) *Synseeds: Applications of Synthetic Seeds to Crop Improvement*. Boca Raton: CRC Press.

Redenbaugh K, Viss P, Slade D, and Fujii J-A (1987) Scale-up: artificial seeds. In: Green CE, Sommers DA, Hackett WP, and Biesboer DD (eds) *Plant Tissue and Cell Culture*, pp. 473–493. New York: Alan R. Liss.

Redenbaugh K, Fujii J-A, and Slade D (1991) Synthetic seed technology. In: Vasil IK (ed.) *Scale-Up and Automation in Plant Propagation*, pp. 35–74. New York: Academic Press.

Roberts DR, Webster FB, Cyr DR, *et al.* (1995) A delivery system for naked somatic embryos of interior spruce. In: Aitken-Christie J, Kozai T, and Smith MAL (eds) *Automation and Environmental Control in Plant Tissue Culture*, pp. 245–256. Boston: Kluwer Academic Publishers.

Sakamoto Y, Onishi N, and Hirosawa T (1995) Delivery systems for tissue culture by encapsulation. In: Aitken-Christie J, Kozai T, and Smith MAL (eds) *Automation and Environmental Control in Plant Tissue Culture*, pp. 215–244. Boston: Kluwer Academic Publishers.

Timmis R (1998) Bioprocessing for tree production in the forest industry: conifer somatic embryogenesis. *Biotechnology Progress* 14: 156–166.

Uozumi N and Kobayashi T (1995) Artificial seed production through encapsulation of hairy root and shoot tips. In: Bajaj YPS (ed.) *Biotechnology in Agriculture and Forestry,* Vol. 30, *Somatic Embryogenesis and Synthetic Seed I*, pp. 170–180. Berlin: Springer-Verlag.

Secondary Metabolism in Plant Cell Cultures

A M Nuutila and K-M Oksman-Caldentey, VTT Biotechnology, Espoo, Finland

Introduction

Plant cell cultures have been investigated for more than 30 years as a potential source of secondary metabolites to rival extraction processes based on whole plant material. However, success so far has been limited. Many questions still remain unanswered, with a particular need for enhanced productivities in cell cultures and simplified and improved process technology. Metabolite engineering might offer new possibilities to enhance the production in cultured cells. The connection between the expression of secondary metabolism and the