Artificial Seeds and their Applications

G V S Saiprasad

G V S Saiprasad is a scientist in Division of Biotechnology, Indian Institute of Horticulture Science, Bangalore. He is currently working on molecular events during ripening in mango and banana and on molecular markets.

Plant propagation using artificial or synthetic seeds developed from somatic and not zygotic embryos opens up new vistas in agriculture. Artificial seeds make a promising technique for propagation of transgenic plants, non-seed producing plants, polyploids with elite traits and plant lines with problems in seed propagation. Being clonal in nature the technique cuts short laborious selection procedure of the conventional recombination breeding and can bring the advancements of biotechnology to the doorsteps of the farmer in a cost-effective manner.

Need for Artificial or Synthetic Seed Production Technology

Development of micropropagation techniques will ensure abundant supply of the desired plant species. In some crop species seed propagation has not been successful. This is mainly due to heterozygosity of seed, minute seed size, presence of reduced endosperm and the requirement of seed with mycorrhizal fungi association for germination (eg. orchids), and also in some seedless varieties of crop plants like grapes, watermelon, etc. Some of these species can be propagated by vegetative means. However, *in vivo* vegetative propagation techniques are time consuming and expensive. Development of artificial seed production technology is currently considered as an effective and efficient alternate method of propagation in several commercially important agronomic and horticultural crops. It has been suggested as a powerful tool for mass propagation of elite plant species with high commercial value. Characteristics of clonal propagation systems are discussed in *Box* 1.

Artificial seed technology involves the production of tissue culture derived somatic embryos encased in a protective coating. Artificial seeds have also been often referred to as synthetic

Box 1. Characteristics of Clonal Propagation Systems

Micropropagation

Low volume, small scale propagation method
Maintains genetic uniformity of plants
Acclimatisation of plantlets required prior to field planting
High cost per plantlet
Relatively low multiplication rate

Greenhouse cuttings

Low volume, small scale propagation method
Maintains genetic uniformity of plants
Rooting of plantlets required prior to field planting
High cost per plantlet
Multiplication rate limited by mother plant size

Artificial seeds

High volume, large scale propagation method
Maintains genetic uniformity of plants
Direct delivery of propagules to the field, thus eliminating transplants
Lower cost per plantlet
Rapid multiplication of plants.

seeds. However, the term 'synthetic seed' should not be confused with commercial seeds of a synthetic cultivar which is defined as an advanced generation of an open pollinated population composed of a group of selected inbred clones or hybrids. The concept of artificial or synthetic seed is shown in *Figure* 1.

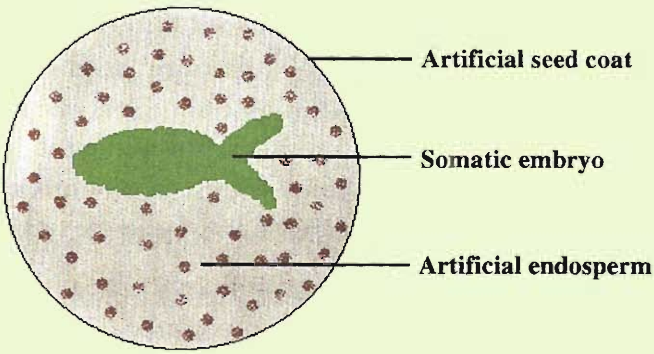

Figure 1. Artificial seed concept.

Box.2. Advantages of Artificial or Synthetic Seeds over Somatic Embryos for Propagation

Ease of handling while in storage
Easy to transport
Has potential for long term storage without losing viability
Maintains the clonal nature of the resulting plants
Serves as a channel for new plant lines produced through biotechnological advances to be delivered directly to the greenhouse or field
Allows economical mass propagation of elite plant varieties.

These synthetic seeds would also be a channel for new plant lines produced through biotechnological advances to be delivered directly to the greenhouse or field. Advantages of artificial/synthetic seeds over somatic embryos for propagation are listed in *Box* 2. This synthetic seed production technology is a high volume, low-cost production technology. High volume propagation potential of somatic embryos combined with formation of synthetic seeds for low-cost delivery would open new vistas for clonal propagation in several commercially important crop species.

What are Somatic Embryos?

Somatic embryos are bipolar structures with both apical and basal meristematic regions, which are capable of forming shoot and root, respectively. A plant derived from a somatic embryo is sometimes referred to as an 'embling'.

Somatic Embryos vs Zygotic Embryos and their Advantages

Somatic embryos are structurally similar to zygotic embryos found in seeds and possess many of their useful features, including the ability to grow into complete plants. However, somatic embryos differ in that they develop from somatic cells, instead of zygotes (i.e., fusion product of male and female gametes) and thus, potentially can be used to produce duplicates of a single genotype. Since the natural seed develops as a result of a sexual process in cross-pollinating species, it is not genetically identi-

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cal to one single parent. In contrast, somatic embryo develops from somatic cells (non-sexual) and does not involve sexual recombination. This characteristic of somatic embryos allows not only clonal propagation but also specific and directed changes to be introduced into desirable elite individuals by inserting isolated gene sequences into somatic cells. This bypasses genetic recombination and selection inherent in conventional breeding technology. If the production efficiency and convenience comparable to that of a true seed are achieved, somatic embryos can be potentially used as a clonal propagation system.

The lack of synchrony of somatic embryos is, arguably, the single most important hurdle to be overcome before advances leading to widespread commercialization of synthetic seeds can occur. Synchronized embryoid development is required for the efficient production of synthetic seeds.

Basic Requirement for Production of Artificial Seeds

Recently, production of synthetic seeds by encapsulating somatic embryos has been reported in few species. One prerequisite for the application of synthetic seed technology in micropropagation is the production of high-quality, vigorous somatic embryos that can produce plants with frequencies comparable to natural seeds. Inability to recover such embryos is often a major limitation in the development of synthetic seeds. Synthetic seed technology requires the inexpensive production of large numbers of high quality somatic embryos with synchronous maturation. The overall quality of the somatic embryos is critical for achieving high conversion frequencies. Encapsulation and coating systems, though important for delivery of somatic embryos, are not the limiting factors for development of synthetic seeds.

At present, the characteristic lack of developmental synchrony in embryogenic systems stymies multi-step procedures for guiding somatic embryos through maturation. The lack of synchrony of somatic embryos is, arguably, the single most important hurdle to be overcome before advances leading to widespread commercialization of synthetic seeds can occur. Synchronized embryoid development is required for the efficient production of synthetic seeds.

Procedure for Production of Artificial Seeds

Establish somatic embryogenesis
↓
Mature somatic embryos
↓
Synchronize and singulate somatic embryos
↓
Mass production of somatic embryos
↓
Standardization of encapsulation
↓
Standardization of artificial endosperm
↓
Mass production of synthetic seeds
↓
Greenhouse and field planting

Types of Gelling Agents used for Encapsulation

Alginate hydrogel is frequently selected as a matrix for synthetic seed because of its moderate viscosity and low spinnability of solution, low toxicity for somatic embryos and quick gellation, low cost and bio-compatibility characteristics.

Several gels like agar, alginate, polyco 2133 (Bordon Co.), carboxy methyl cellulose, carrageenan, gelrite (Kelko. Co.), guargum, sodium pectate, tragacanth gum, etc. were tested for synthetic seed production, out of which alginate encapsulation was found to be more suitable and practicable for synthetic seed production. Alginate hydrogel is frequently selected as a matrix for synthetic seed because of its moderate viscosity and low spinnability of solution, low toxicity for somatic embryos and quick gellation, low cost and bio-compatibility characteristics. The use of agar as gel matrix was deliberately avoided as it is considered inferior to alginate with respect to long term storage. Alginate was chosen because it enhances capsule formation and also the rigidity of alginate beads provides better protection (than agar) to the encased somatic embryos against mechanical injury. Alginate encapsulated somatic embryos of orchids are shown in *Figure* 2 and the plantlets derived from artificial or synthetic seeds of orchid are shown in *Figure* 3.

Figure 2 (left). Artificial or synthetic seed produced in orchids by alginate encapsulation.
Figure 3 (right). Artificial or synthetic seed derived plantlets in orchid.

Principle and Conditions for Encapsulation with Alginate Matrix

Alginate is a straight chain, hydrophilic, colloidal polyuronic acid composed primarily of hydro-β-D-mannuronic acid residues with 1-4 linkages. The major principle involved in the alginate encapsulation process is that the sodium alginate droplets containing the somatic embryos when dropped into the $CaCl_2.2H_2O$ solution form round and firm beads due to ion exchange between the Na^+ in sodium alginate with Ca^{2+} in the $CaCl_2.2H_2O$ solution. The hardness or rigidity of the capsule mainly depends upon the number of sodium ions exchanged with calcium ions. Hence, the concentration of the two gelling agents i.e., sodium alginate and $CaCl_2.2H_2O$, and the complexing time should be optimized for the formation of the capsule with optimum bead hardness and rigidity. In general, 3% sodium alginate upon complexation with 75 mM $CaCl_2.2H_2O$ for half an hour gives optimum bead hardness and rigidity for the production of viable synthetic seeds.

Artificial Endosperm

Somatic embryos lack seed coat (testa) and endosperm that provide protection and nutrition for zygotic embryos in developing seeds. To augment these deficiencies, addition of nutrients and growth regulators to the encapsulation matrix is desired, which serves as an artificial endosperm. Addition of nutrients and growth regulators to the encapsulation matrix

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results in increase in efficiency of germination and viability of encapsulated somatic embryos. These synthetic seeds can be stored for a longer period of time even up to 6 months without losing viability, especially when stored at 4 °C.

Addition of Adjuvants to the Matrix

In addition to preventing the embryo from desiccation and mechanical injury, a number of useful materials such as nutrients, fungicides, pesticides, antibiotics and microorganisms (eg. rhizobia) may be incorporated into the encapsulation matrix. Incorporation of activated charcoal improves the conversion and vigour of the encapsulated somatic embryos. It has been suggested that charcoal breaks up the alginate and thus increases respiration of somatic embryos (which otherwise lose vigour within a short period of storage). In addition, charcoal retains nutrients within the hydrogel capsule and slowly releases them to the growing embryo.

Utilization of Artificial Seeds

The artificial seeds can be used for specific purposes, notably multiplication of non-seed producing plants, ornamental hybrids (currently propagated by cuttings) or the propagation of polyploid plants with elite traits. The artificial seed system can also be employed in the propagation of male or female sterile plants for hybrid seed production. Cryo-preserved artificial seeds may also be used for germplasm preservation, particularly in recalcitrant species (such as mango, cocoa and coconut), as these seeds will not undergo desiccation. Furthermore, transgenic plants, which require separate growth facilities to maintain original genotypes may also be preserved using somatic embryos. Somatic embryogenesis is a potential tool in the genetic engineering of plants. Potentially, a single gene can be inserted into a somatic cell. In plants that are regenerated by somatic embryos from a single transgenic cell, the progeny will not be chimeric. Multiplication of elite plants selected in plant breeding programs via somatic embryos avoids the genetic re-

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combination, and therefore does not warrant continued selection inherent in conventional plant breeding, saving considerable amount of time and other resources. Artificial seeds produced in tissue culture are free of pathogens. Thus, another advantage is the transport of pathogen free propagules across the international borders avoiding bulk transportation of plants, quarantine and spread of diseases.

Potential Uses of Artificial Seeds

Delivery systems:

- Reduced costs of transplants
- Direct greenhouse and field delivery of:

  elite, select genotypes
  hand-pollinated hybrids
  genetically engineered plants
  sterile and unstable genotypes
- Large-scale mono cultures
- Mixed-genotype plantations
- Carrier for adjuvants such as microorganisms, plant growth regulators pesticides, fungicides, nutrients and antibiotics
- Protection of meiotically-unstable, elite genotypes
- Can be conceivably handled as seed using conventional planting equipment.

Analytical tools:

- Comparative aid for zygotic embryogeny
- Production of large numbers of identical embryos
- Determination of role of endosperm in embryo development and germination
- Study of seed coat formation
- Study of somaclonal variation.

Applicability and Feasibility of Artificial Seed Production Technology

In order to be useful, synthetic seed must either reduce production costs or increase crop value. The relative benefits gained, when weighed against development costs, will determine whether its use is justified for a given crop species. Considering a combination of factors, including improvement of the existing embryogenic systems, relative cost of seed as well as specific application for synthetic seed allows judgement of relative need for a given crop. For example, synthetic seed of seedless watermelon would actually cost less than conventional seed, providing a benefit at the outset of crop production. Although embryogenic systems for this crop do not exist, the benefit that could be conferred by use of synthetic seed would be very great. Value-added aspects that would increase crop worth are numerous and include cloning of elite genotypes, such as genetically engineered varieties, that cannot produce true seed.

Suggested Reading

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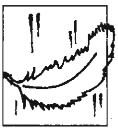
Address for Correspondence
G V S Saiprasad
Division of Biotechnology
IIHR, Hessarghatta Lake Post
Bangalore 560 089, India.



There are grounds for cautious optimism that we may now be near the end of the search for the ultimate laws of nature.

Stephen W Hawking
A brief history of time: from the Big Bang to Black Holes