Artificial Seeds

D J Gray, University of Florida, Apopka, FL, USA

Background

Conventional Seed

Production of most major agronomic crops utilizes seeds for stand establishment. This is due to their overall low cost and efficiency when compared to other methods of planting crops, such as the use of cuttings. With few exceptions, a seed is composed of a zygotic embryo, which is encased in nutritive tissues and several layers of protective coats. Due to the common use of seeds, we tend to take their special properties for granted. Seeds are extremely durable due to the protective coats and are desiccation tolerant, so that they can be dried and become quiescent. These qualities allow seeds to be stored *en masse* at room temperature and sown with relatively simple planting equipment. Due to their ability to become quiescent, seeds also are used for germplasm preservation in seed repositories. The primary disadvantage to the use of seed rests with the origin of the zygotic embryo, which is the result of sexual reproduction; the progeny of two parents. This has led to the development of often complex breeding programs from which inbred parental lines are developed. Such inbred lines are used to produce uniform hybrid progeny when crossed. For many crops, such as fruits, nuts, and certain ornamentals, it is not possible to produce a true-breeding seed from two parents due to genetic barriers to selfing. For other crops, such as forest trees, the generation time is too long to achieve rationally an inbred breeding program. Therefore, for such crops, propagation is accomplished either vegetatively by cuttings or the use of relatively low-quality open pollinated seed is tolerated.

Artificial Seed

For a number of applications, it would be advantageous to combine the efficiency aspects of seed with clonal plant production. The discovery of somatic embryogenesis in the 1950s provided a possible alternative to conventional seed. Somatic embryos are structurally equivalent to zygotic embryos, but are true clones, since they arise from the somatic cells of a single "parent." Somatic embryos differ from zygotic embryos in that they are produced via *in vitro* culture, develop without nutritive and protective seed coatings, and do not typically become quiescent. The field that seeks to use somatic embryos as functional seed is termed "artificial or synthetic seed technology." Thus, artificial seeds are defined from a practical standpoint as somatic embryos engineered to be of use in commercial plant production and germplasm preservation. The actual structural complexity of artificial seed depends on requirements of the specific crop application. Therefore, a functional artificial seed may or may not require a synthetic seed coat, be hydrated or dehydrated, quiescent or nonquiescent, depending on its intended usage.

Structure and Development of Conventional versus Artificial Seeds

Zygotic and Somatic Embryos

Zygotic and somatic embryos share many structural characteristics, with both typically passing through globular, torpedo, and cotyledonary stages for dicots or globular, scutellar, and coleoptilar stages for monocots. They also share a key useful developmental feature in that both are able to form complete plants without separate rooting and shoot development phases. Since somatic embryos develop from somatic cells, instead of zygotes, they differ in that the former can be used to produce duplicates of a single genotype.

Desiccation tolerance and quiescence Desiccation tolerance and onset of quiescence, which are key developmental mechanisms leading to the usefulness of zygotic embryos in conventional seed, are either missing or have been overlooked in somatic embryos. Zygotic embryos in conventional seed begin to lose water content and enter a resting period during maturation which is the major factor allowing them to be conveniently stored. Basic developmental mechanisms that induce or allow the resting phase differ among species and have been variously categorized. Generally, two broad categories of arrested growth, "quiescence" and "dormancy," are distinguished. Quiescence is a resting phase that can be reversed solely by the addition of water. Dormancy is a form of quiescence that requires factors in addition to water, such as cold or heat treatments, for resumption of growth to occur. Typically, quiescent seed can be kept for prolonged time periods without a noticeable loss of viability. Seed germplasm repositories utilize specific temperature/relative humidity regimes to optimize long-term viability.

In contrast to resting zygotic embryos in seeds, somatic embryos typically do not become quiescent; rather, they tend to continue to grow and typically either germinate, become disorganized into embryogenic tissue, or die. Lack of a resting phase in somatic embryos is a major drawback to their use as artificial seed. Somatic embryos with reversible, arrested growth (i.e., quiescence) will be needed in order to mimic seed storage and handling characteristics.

Dormancy, wherein embryo proliferation occurs but not germination and plant development, has been documented in somatic embryos of several species and may be unrecognized in those of others. Certain types of dormancy, such as those regulated by nonembryonic seed tissues, clearly cannot be a factor in existing embryogenic culture systems. However, mechanisms of quiescence and dormancy that are controlled by water availability, temperature, light, inhibitors, promoters, etc., may be functional in somatic embryos of species with corresponding zygotic embryo dormancy. Quiescence has been induced experimentally in somatic embryos of several species, usually by controlled dehydration, often combined with modification of culture nutrients (such as increase of carbohydrate sources) and plant growth regulators (such as addition of abscisic acid (ABA)).

To experimentally induce quiescence in (normally) rapidly growing somatic embryos, lowering of water content by dehydration is a logical approach since dehydration and rehydration cause arrest and resumption of growth in orthodox seeds. The first example of induced quiescence in somatic embryos was documented in orchardgrass (*Dactylis glomerata*). Somatic embryos were dehydrated in a 70% RH environment and survived up to 21 days at 23°C. The embryos became discolored, decreased in size, and their outer cell walls collapsed. Embryo water content was reduced from 83% to 13% within 24 h. This water content is similar to that of seeds maintained at 70% RH and is adequate for maintenance of viability during prolonged seed storage. During rehydration, the embryos regained their normal appearance and germinated. However, only mature embryos which contained starch and lipid storage compounds survived. Of mature dehydrated somatic embryos stored for 21 days, 4% produced plants after imbibition. This study convincingly demonstrated that quiescence could be induced to occur in somatic embryos.

Grape (*Vitis vinifera*) somatic embryos were used to first demonstrate quiescence in a dicotyledonous species. They were treated similarly to orchardgrass and quiescence was induced. In grape, genotypic differences in response were noted; those varieties that produced relatively well-developed somatic embryos in culture were most responsive. After 21 days, 34% of dehydrated somatic embryos from one genotype produced plants following imbibition. More recently, somatic embryos of grape, dehydrated to 25% of original moisture content, exhibited 90% viability after 42 months of storage at 6°C. Such somatic embryos were capable of germination and plant development. This study demonstrated that high germination percentages and long storage periods are possible.

Quiescence has also been observed in somatic embryos of corn (*Zea mays*, maize) and soybean (*Glycine max*). Approximately 90–100% selected, high-quality somatic embryos of alfalfa (*Medicago sativa*, lucerne), produced plants when treated with ABA before drying. After 1 year of dried storage without humidity control, 60% of the embryos germinated into seedlings. The ability of alfalfa embryos to survive dehydration was thought to be due to addition of ABA; this appeared to induce desiccation tolerance. Thus, ABA-treated somatic embryos of alfalfa mimicked typical quiescent seed. Further refinements to the system showed that $10\,\mu\mathrm{mol\,l}^{-1}$ ABA applied to 14-day-old embryos was most effective. Slow drying was more effective than rapid drying.

In somatic embryos, timed application of ABA and controlled drying appear to coincide with the developing seed environment. Induction of desiccation tolerance in somatic embryos shows that environmental conditions regulate whether or not they assume the complex developmental pathways normally associated with those of zygotic embryos in seed.

Nutritive and Seed Coat Tissues

Tissues surrounding zygotic embryos in seed serve to provide nutrition and/or protection. A major distinction between zygotic and somatic embryos is that the latter develop without these tissues. In addition, they create barriers to regulate gas exchange. For example, protective tissues limit oxygen availability, which influences respiration and embryo development and prevents precocious germination. For somatic embryos, the culture vessel in which they are grown determines the available gas regime. Availability of oxygen and other gases depends on subculture interval, whether or not the vessel is tightly sealed, as well as gas usage and evolution by other associated tissues, such as subtending callus. This is a stark contrast to the highly regulated and developmentally controlled seed environment. In fact, immature zygotic embryos often do not develop normally if removed prematurely from the seed and placed onto culture medium; this reinforces the role of seed tissues in providing the proper environment for development.

Embryo nutrition: zygotic embryos Within seeds, the tissue that performs a nutritive function is the endosperm or female gametophyte in angiosperms and gymnosperms, respectively. Nucellar tissue also performs a nutritive function in many seeds. All substances required by the embryo must pass either through the suspensor or directly from the encasing nutritive tissues. Relative dependence on suspensor versus endosperm for nutrition varies between species. Depending on species, endosperm may or may not persist in the mature seed to provide nutrition during germination. The composition of storage substances also differs among species but consists predominantly of carbohydrates, lipids, and proteins. Endosperm may also function to regulate embryo water balance. Embryos initially develop with a suspensor connection; however, those with endospermic seeds, such as tobacco (*Nicotiana tabacum*), shift from suspensorial to endosperm nutrition at some point in their development. Such embryos are able to utilize nutrients from endosperm at an early stage. In nonendospermic seeds, such as cucurbits and legumes, endosperm either does not form in appreciable amounts or is exhausted during development so that the mature embryo must contain adequate storage reserves for germination.

Germination requires energy reserves stored either in endosperm (endospermic seeds) or the embryo itself (nonendospermic seeds). Storage reserves are mobilized during germination. The complex developmental mechanisms that lead to the accumulation then usage of storage compounds are partially responsible for the long-term viability and rapid germination qualities of seed.

Embryo nutrition: somatic embryos The ability of somatic embryos to develop in a manner that parallels that of zygotic embryos is surprising considering the significant differences in the environments in which they develop. Zygotic embryos of a given species are adapted to grow in very species-specific and complex nutrient environments. Whereas, with somatic embryos, there is no endosperm tissue to feed the developing embryo and modulate nutrient flow. Somatic embryos develop in a highly unorganized manner on or within callus cultures or immersed in liquid medium. In fact, only a few commonly available culture media have been used to produce somatic embryos from hundreds of different species. In retrospect, this is rather unexpected, considering the large range of nutrient regimes among various seed types that these reports parallel. Often, a suspensor is the only link between the embryo and growth medium. Interestingly, somatic embryos commonly develop perched above subtending callus or explant tissue on narrow suspensors without any opportunity for nutritive factors to be absorbed by the embryo body. This demonstrates that the suspensor can serve as the pathway to provide all needed nutrition and that endosperm is not necessary for embryo development. However, it is likely that these significant differences in the environments between zygotic and somatic embryos result in the irregularities noted with the latter.

Protective coatings: zygotic embryos Seed coats are composed of structurally complex lignified tissues that originated from the floral ovule and, for seed pods, the ovary. Seed coats variously fulfill a number of functions. Often, there are structured openings through the coats to permit the entrance of water to facilitate germination. Some seed coats possess accessory tissues that constitute “wings” or other structures, such as hinges that spring open with drying to facilitate dispersion. They provide protection from disease-causing organisms and mechanical stress. They also function to preserve dormancy in some species by a variety of mechanisms, including exclusion of water. The seed coat provides a limited amount of space in which the embryo and accessory tissue must develop; this often causes the embryo to become compressed and/or flattened into a shape and size distinct for a given species or variety. In comparison, corresponding somatic embryos tend to be larger and not flattened, with wider hypocotyls and fleshier cotyledons. The force exerted by the developing embryo within the seed coat also determines pressure potential, thus affecting osmotic potential and water content.

Protective coatings: somatic embryos Artificial seed coats have been demonstrated. However, mimicking certain of the complex functions of seed is not possible and probably not necessary. Specialized dispersal structures are not needed, since artificial seed will be handled with mechanical planting devices. Seed coat-induced pressure to alter morphology and water content of somatic embryos, while possibly preferable in producing high-quality analogs to conventional seed, may be difficult to achieve.

Artificial seed coats consisting of plastic resins, organic ingredients, or other materials have been demonstrated. However, calcium alginate, which forms a gel, has been used in most examples. Additives, including starches, sugars and antimicrobial substances like fungicides, have been incorporated into the coating. Functionally, such coatings then constitute an artificial endosperm and seed coat in one structure when used to encapsulate an embryo. Both hydrated and dehydrated artificial seed coats have been tested.

To date, most examples of artificial seed coats have not shown adequate utility. In nearly all instances, somatic embryos encapsulated in such coatings lose viability faster than embryos that are not encapsulated. Nevertheless, as described below, protective and/or nutritive coatings will be required for certain practical implementations of artificial seed technology; hence, research into their development is urgently needed.

The foregoing discussion of nutritive and seed coat tissues has underscored significant differences in physical environment that occur between somatic embryos and zygotic embryos. Both somatic and zygotic (in seed) embryos placed on an appropriate germination medium are connected to the medium by a liquid interface. However, only the somatic embryo is directly exposed to medium. The zygotic embryo is separated from medium by seed coatings and endosperm, although a direct channel to the outside may occur in some types of seed. Thus, somatic embryos develop and germinate in an environment unlike that of zygotic embryos. Certain aspects of the complex seed environment, such as presence of an array of polysaccharides, minerals, proper pH, etc., are obviously necessary for development of normally germinating somatic embryos and are provided in commonly used culture media. However, since zygotic embryos excised from seed and placed on nutrient medium often do not develop properly and tend to exhibit abnormalities common to somatic embryos, it is probable that additional modifications will be necessary. Some of the nutrient factors in seeds such as specific polysaccharides and amino acids can be readily incorporated into culture medium and have been used in a number of systems, including alfalfa and Norway spruce (*Picea abies*). However, simulating complex nutrient pulses and the precise physical environment created in seed will be more difficult.

Types of Artificial Seed

Depending on the specific application and production needs of a given crop, functional artificial seed can assume several different forms. The somatic embryo may be quiescent or nonquiescent and a protective coating may or may not be present. For example, uncoated nonquiescent somatic embryos could be used to produce those crops that are now laboriously micropropagated by tissue culture. Manpower reduction and, hence, cost reduction achieved by producing plants by somatic embryogenesis would be significant compared to that of micropropagation. Uncoated, quiescent (dried) somatic embryos would be useful for germplasm storage since they can be hand-stored in existing seed storage repositories. Cost of manipulating somatic embryos for such storage would be similar to that of seed but would constitute a clonal source of germplasm.

Nonquiescent somatic embryos placed in a hydrated encapsulation constitute a type of artificial seed that may be cost effective for certain field crops that pass through a greenhouse transplant stage such as carrot (*Daucus carota*), celery (*Apium graveolens*), seedless watermelon (*Citrullus vulgaris*), and other vegetables. However, if long-term storage is not necessary, artificial seed production and planting would have to be closely coordinated.

Artificial seeds consisting of quiescent encapsulated somatic embryos could be more useful than the nonquiescent form, since the artificial seed production phase could be separated from the plant production phase.

Dehydrated, quiescent somatic embryos encapsulated in artificial coatings are the form of artificial seed that most resembles conventional seed in storage and handling qualities. These consist of somatic embryos encased in artificial seed coat material, which then is dehydrated. Under these conditions, the somatic embryos become quiescent and the coating hardens. Theoretically, such artificial seeds are durable under common seed storage and handling conditions. Upon rehydration, the seed coat softens, allowing the somatic embryo to resume growth, enlarging and emerging from the encapsulation. This type of synthetic seed would be necessary for field planting using conventional technology. Unfortunately, there has not been sufficient development of the dehydrated, encapsulated form of artificial seed to allow commercialization. Since dehydrated uncoated somatic embryos can survive significantly longer storage periods, it appears that the materials currently used as artificial seed coats are detrimental to survival. Additional research is required to develop more acceptable coatings.

In addition to application of an artificial seed coat to facilitate planting, other methods of delivering somatic embryos to soil, such as fluid drilling, have been proposed.

Applications for Artificial Seeds

The particular usage of artificial seed for a given crop will depend on several factors, including specific production needs that are not met with existing systems. For artificial seed to be of commercial use, crop production must ultimately be more profitable than with existing systems. Various crops are discussed below to illustrate possible applications of different forms of artificial seed.

Ornamental Crops

Many ornamental crops already are propagated via tissue culture where per-plant production costs are relatively high due to manpower requirements. Micropropagation of shoot cultures is routinely used; this process requires multiple laborious handling steps to ultimately produce rooted plants. Use of artificial seed, in this form consisting of naked hydrated embryos, would alleviate much of the manpower requirements. Somatic embryos mass-produced in culture could be selected and placed directly into planting flats, resulting in rooted plants and thus eliminating several labor-intensive steps.

Fruit and Nut Crops

Most fruit and nut crops currently are economically propagated clonally from cuttings due to genetic self-incompatibility and long breeding cycles. While artificial seed could possibly be used to facilitate production, existing methods are cost effective and development costs of the technology may not be justified. Efficiency aspects of artificial seed may be further reduced if the resulting clones require grafting, as do many existing fruit and nut varieties. However, use of dehydrated, quiescent, uncoated forms of artificial seed could be of significant use in germplasm conservation, since they could be stored in conventional seed repositories. This would alleviate the expensive reliance on field gene banks, in which plants are kept in field repositories where they are subject to loss from environmental disasters.

Vegetable Crops

Artificial seed could provide a cost advantage to those vegetable crops that currently have high seed costs and high per-plant value. For example, many hybrid varieties are very expensive to produce. Some are produced as transplants in greenhouses before transfer to the field. For seedless watermelon, the combination of high seed cost, due to barriers to seed production, and low germination rates can result in transplants costing US$1.00 each. For seedless watermelon, artificial seed could reduce per-plant costs by circumventing barriers to seed production. Artificial seed, in this case naked or encapsulated hydrated somatic embryos, could be treated in the same manner as conventional seed in transplant production.

Hybrid Cereals

Another application of artificial seed would be to remove the reliance on inbred and male-sterile parental lines in hybrid seed production. For example, the hybrid corn industry relies on inbred parentals to produce uniform hybrid seed. Mass seed production is made possible by use of male-sterile lines as females. Resulting hybrid plants exhibit increases in yield and quality; however, development of true-breeding parental lines is extremely time-consuming and costly. As an alternative, artificial seed could be used to clone and mass-produce one outstanding hybrid, eliminating the most demanding parts of a hybrid program. For this usage, artificial seed would have to function as true analogs of conventional seed in planting and production so that existing equipment could be used.

Conifers in Forestry

Due to relatively low per-plant value (when value is amortized over production time) and high volume of plants needed, seed must be used to establish stands of conifers. Conifers are relatively unimproved genetically due to barriers associated with the long reproductive life cycle and extended time needed to evaluate progeny. Therefore improvement via conventional breeding is extremely slow. Ability to select existing outstanding individuals from the forest and clone them economically as artificial seed would allow rapid progress in tree quality to be achieved. The form of artificial seed most amenable to forest tree production would be dehydrated, quiescent somatic embryos, encapsulated in a protective coating. This would allow artificial seed to be utilized in conventional production systems.

Conclusion

To date, the greatest progress in implementation of artificial seed technology has been with conifers. Production systems in place today allow the efficient establishment in soil of thousands of trees at a time. However, development of similar systems for other crops has been slow. This may be due to the lack of a driving need for improvement, since existing systems tend to be profitable in light of the relatively large amount of investment capital needed to produce true artificial seed (i.e., commercially useful somatic embryos, as defined here). Advances in the state-of-the-art of embryogenic cell culture are continuing and it is likely that certain improvements eventually will actualize the use of artificial seed for additional crop applications.

List of Technical Nomenclature

Abscisic acid (ABA)	A plant growth regulator associated with embryo maturation and quiescence.
Artificial seed	A somatic embryo used in commercial plant production or germplasm preservation.

Artificial seed coat Protective man-made material used to coat, protect and/or provide nutrition to somatic embryos.

Desiccation tolerance Ability of embryos to survive dehydration.

Dormancy Resting phase in embryos in which reversal requires factors in addition to water.

Endosperm Nutritive tissue of zygotic origin, which encases and provides nutrition to zygotic embryos.

Quiescence Resting phase of embryos usually reversed by addition of water.

Seed coat Protective tissue of maternal origin surrounding zygotic embryos for protection.

Somatic embryo Clonal embryo resulting from *in vitro* cell culture; the duplicate of one "parent."

Suspensor Organ connecting developing embryo to subtending tissue.

Zygotic embryo Embryo resulting from sexual reproduction; the progeny of two parents.

See also: **Crop Improvement**: Chromosome Engineering. **Postharvest Physiology**: Seed Storage. **Seed Development**: Embryogenesis; Germination; Seed Production. **Tissue Culture**: Somatic Embryogenesis.

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Further Reading

Bewley JD and Black M (1985) *Seeds: Physiology of Development and Germination*. New York: Plenum Press.

Gray DJ (1987) Introduction to the symposium. In: Proceedings of the Symposium on synthetic seed technology for the mass cloning of crop plants: problems and perspectives. *HortScience* 22: 796.

Gray DJ and Purohit A (1991) Somatic embryogenesis and development of synthetic seed technology. *Critical Reviews in Plant Sciences* 10: 33–61.

Ragahavan V (1986) *Embryogenesis in Angiosperms*. New York: Cambridge University Press.

Trigiano RN and Gray DJ (2000) *Plant Tissue Culture Concepts and Laboratory Exercises*, 2nd edn. Boca Raton: CRC Press.

Seed Production

R E L Naylor, Trelareg Consultants, Banchory, Scotland, UK

Introduction

Seeds are a basic resource in agriculture. They represent the foundation and continuation of all crops. The grower requires that the seeds sown fulfill the basic requirement of being capable of producing a good plant stand. The seeds thus have to be alive and capable of germination without the delay imposed by prolonged dormancy. The producer of these seeds therefore has to pay particular attention to fostering all the biological processes involved in seed production and to minimize any detrimental influences on subsequent germination.

In some cases, seeds are still collected from the wild, albeit from specific locations. An example is forest trees in many of which geographic provenances or specific seed trees may be identified with the aim of restricting the genetic variability in the established plants. In field crops, the seeds may themselves be the agricultural product (e.g., cereals, mustard, coconut) in which case there may be additional quality criteria determined by the end use of what is then an agricultural product. Crops in which the seed is not the main product, e.g., crops produced for their leaves (lettuce, grass) or their stems (flax, sugar cane) or their roots (sugar beet, carrots) are likely to require a different agronomy if seed production is the aim of growing the crop. Even in clonally produced crops (e.g., potatoes) sexual reproduction and seed production are essential for the generation of new cultivars. The major focus of this article is on producing seed for sowing the next crop.

Seed production is generally preceded by pollination and starts with the fertilization of the ovule. It can be considered as ending when the seed is shed from the parent plant or is harvested. After-ripening, seed storage conditions, and handling all play a role in determining seed quality at planting. This article will summarize the biological processes involved in seed production and then use this to develop the principles of seed production agronomy. It will examine the impacts of various sorts of "stress" on seed production. The focus will be on conventional seeds; species with recalcitrant seeds will not be considered. Seed storage after harvest will not be considered.