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CURRENT STATUS AND FUTURE PROSPECTS OF ARTIFICIAL SEEDS

Jo Ann A. Fujii, David Slade, and Keith Redenbaugh¹

Introduction

The main concept of artificial seeds is to deliver tissue-cultured plants to the greenhouse or field environment. While micro-propagation techniques allow for mass propagation and cloning of selected plants, the cloned plantlets must be acclimated prior to introduction into the greenhouse or field. With artificial seeds, somatic embryos produced through tissue culture can be directly delivered to the soil environment without the formation of transplants which may require an acclimation step prior to field planting. This would allow for the distribution of clonal elite plant material without the laborious and expensive task of transplant production. The use of artificial seeds could decrease the cost of tissue-cultured material by providing a channel for the introduction of novel plants that cannot be distributed using botanic seeds. The agricultural impact of such a delivery system would be extensive as it would interface emerging cell biology/tissue culture technology with greenhouse/field production systems.

Artificial seeds, in the truest definition, consist of single somatic embryos in a protective coating that mimic the size and shape of true seeds. In this discussion we will focus on the general concepts of artificial seeds for the mass propagation and delivery of plants.

In reviewing the current status and future prospects of artificial seed technology, we will begin with an overview of the future followed by a discussion of the current status, which is heavily driven by anticipated future benefits of the system. A discussion of the types of artificial seed systems currently under research and the productions and components of a hydrated artificial seed system will be reviewed using, as a model, the alfalfa artificial seed system developed at Plant Genetics, Inc. We will conclude with a review of what we've learned about artificial seeds thus far and what

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issues need further focus to achieve a viable commercial artificial seed system.

Background

The artificial seed concept was first discussed in the early to mid-1970's. However, it was not until 1979-1980 that data were published on utilizing somatic embryos in an artificial seed delivery system (Drew, 1979; Kitto and Janick, 1980).

The potential benefits of using an artificial seed propagation system are numerous (Table 1). As we continue to make progress in artificial seed research, we will have a clearer focus on which characteristics are most essential for a successful system. Although the list of benefits for using artificial seeds is substantial, the commercial potential of the system will rely heavily on the specific value of the propagated crop and the cost of competing products and technologies. The potential use of artificial seeds will also depend on technological developments in other plant science areas, such as the development of superior crop lines through genetic engineering. However, we can currently identify some examples of crops that have potential to benefit greatly from an artificial seed system (Table 2). These examples are focused on markets where true seeds are unavailable and a high cost per artificial seed could, therefore, be supported by market demand.

While an artificial seed system would be useful in various crop situations, there are many technological problems that need to be overcome (Table 3). The issue of somaclonal variation, which is plant-to-plant variation due to in vitro culture, is a major obstacle for crops where uniformity is extremely important (D'Amato, 1978). Even after the target crop is identified, the somatic embryogenesis is often not understood or well characterized as, in general, the control and regulation of most somatic embryogenesis processes have not been obtained. To bypass many of these problems, investigators have chosen to use model crops that possess a well-defined and controllable somatic embryogenesis system but do not possess any commercial value for propagation through an artificial seed system. Therefore, most, if not all, artificial seed development has been conducted using species that do not have a directly commerciable artificial seed system, such as with carrot and alfalfa. Although these model systems will not lead directly to salable products, they will provide evidence for proof-of-concept and, therefore, stimulate continued support for research.

Additional potential drawbacks for artificial seeds include the consistent and predictable production of vigorous somatic embryos, which are essential for the recovery of viable plants. Currently very little is understood about the in vitro conditions that control and

Table 1. Potential benefits of an artificial seed delivery system.

- Rapid propagation of desirable crop lines
- Genetic uniformity of plants
- Direct delivery of tissue cultured plants to the field, thus eliminating transplant propagule
- Deliver genetically unique genotypes, such as genetically engineered plant lines, genotypes which cannot be propagated by seed, hybrids where no cost effective production system exists, and meiotically unstable genotypes
- Deliver sexually sterile crops
- Reduce cost of vegetatively propagated elite lines

Table 2. Crops that may benefit from an artificial seed delivery system.

- Hybrid rice
 - Requires rapid propagation of new F₁ hybrids
 - Potato
 - Due to genetic instability, true seeds are not used for propagation
 - Propagation is by cut tuber pieces that are prone to disease infection
 - Low storability of tuber pieces a major problem in tropical regions
 - Geraniums
 - True seed costs are high and is currently vegetatively propagated
 - European seedless cucumber
 - True seeds are expensive (27 cents/seed)
 - Garlic
 - Currently vegetatively propagated using cloves. There is a high carryover of virus and nematodes to subsequent generations.
 - Gerbera Daisy
 - High cost of seeds (14 cent/seed)
-

Table 3. Potential drawbacks of artificial seeds.

- Somaclonal variation

High levels of somaclonal variation reported in various crops with very little understanding of factors that reduce variation

- Embryo quality and vigor

Lack of understanding and control over somatic embryo quality and subsequent plant vigor

- Somatic embryogenesis in desirable genotypes

Lack of controlled, well-defined somatic embryogenesis in high-value, commercial plant lines

- Artificial seed storage

Lack of knowledge on storability and maintenance of viability of somatic embryos over extended periods

promote the production of high quality embryos. Also, the conditions that maintain embryo vigor, even through long storage periods, have not been addressed.





Current Status

Types of Artificial Seed Systems

There are four types of artificial seed systems currently under research. These systems range from hydrated embryos in a gel capsule or a fluid drilling matrix, to desiccated embryos in a dried wafer (Figure 1). Each artificial seed system has specific advantages and disadvantages inherent in the system. The hydrated embryo encapsulation system, consisting of a single somatic embryo in a gel capsule, may be the most useful for the delivery of single embryos in crops that need to be precision planted. However, the singulation and encapsulation process needs to be automated and this may pose a technical hurdle. Fluid drilling allows for the mass handling of large quantities of embryos, yet fluid drilling is unsuitable for precision planted crops and special fluid drilling planters must be used. Desiccated somatic seeds allow for ease of storage and transport but require additional desiccation steps beyond the hydrated embryo systems. Although the physical appearance of each artificial seed system has differed greatly, the common research goal is to efficiently deliver somatic embryos to the germination environment and to recover whole plants from those embryos. Achieving this goal will require research into both somatic embryogenesis and embryo coating materials. The most important aspect of the artificial seed system may be the interaction between these two research areas to form a artificial seed system.

The current literature on artificial seed research reflects a strong bias towards viewing artificial seed development as a basic research investigation. By approaching artificial seed development in this manner, many investigators have not addressed issues relating to commercialization. Most investigators have not begun to estimate the efficiency of the propagation method. A mass balance calculation would be essential to estimate the output of number of plants per unit input of grams of plant tissue. Many investigators have also not begun testing the artificial seed system under production-like conditions such as soil germination in the greenhouse or field. Failure to anticipate how artificial seeds may fit into existing commercial operations, such as in transplant production and mechanization in greenhouses, could hinder the use of artificial seeds by adding additional equipment costs or modifications to the greenhouse conditions.

Figure 1. Research efforts on artificial seeds.

Researcher	Crop	Concept
Gray (1986 _{a,b})	orchardgrass grape	 desiccated, uncoated embryo
Kitto & Janick (1980, 1981, 1982, 1985 _{a,b})	carrot	 desiccated, coated embryo clumps
Redenbaugh et al. (1986 _{a,b})	alfalfa celery	 hydrated, encapsulated embryo
Schultheis (1986)	sweet potato	 hydrated embryos in fluid drilling gels

Thus, while there is a need to focus on specific aspects of artificial seed development (somatic embryogeny, coating or encapsulation for delivery, recovery of plants in soil, etc.), integration of all aspects of artificial seeds is crucial if a commercially feasible product is to be obtained.

Process of Somatic Embryogenesis

Somatic embryos can be produced from various parts of plants. Through the use of plant hormones and specific nutrients, these plant parts can be induced to form unorganized cell clusters or callus. Additional hormone treatments will further induce the callus to form somatic embryos. The somatic embryos produced through cell culture are developmentally and biochemically similar to zygotic embryos.

The research efforts on alfalfa at Plant Genetics, Inc. have focused on a line of alfalfa that was selected for a high level of somatic embryo production. This alfalfa line has served as a good model system to study not only the somatic embryogenesis process but also to develop the encapsulation and artificial seed planting methods. This alfalfa line produces "high-quality" somatic embryos which are not only morphologically normal but also produce a high percentage of normal plants. The quality of an embryo is, thus, an assessment of both embryo appearance as well as the vigor of the plantlet formed from the embryo. By using strict criteria to evaluate the somatic embryogenesis system, we have optimized our system for both the production of a high number of embryos as well as a high level of conversion of the somatic embryos to plants.

Through identification of medium additives during various stages of alfalfa somatic embryogeny, somatic embryo development (size) and ability to form normal plants *in vitro* has increased (Stuart and Strickland, 1984_{a,b}). In addition, numbers of somatic embryos regenerated per gram callus were significantly increased. The improvements in alfalfa embryo quality resulting from embryogenesis research during 1982-1985 are evidenced by increased frequency of recovery of whole plants, from less than 0.5% recovery in 1982 to 50-60% in 1985 (Redenbaugh, *et al.* 1986_a). The consistent recovery of 50-60% whole plants was a consequence of the increased level of understanding and control over the somatic embryogenesis process in alfalfa.

Production of Hydrated Artificial Seeds

Artificial seed research at Plant Genetics, Inc. has focused on using a hydrated delivery system for somatic embryos. Two patents for the encapsulation of meristematic tissue, such as somatic embryos and shoots, in hydrated gels and gels containing additives have been granted (Redenbaugh 1986_{c,d}). A large number of hydrogels were tested on somatic embryos and true seeds for various parameters such as ease

of capsule formation and lack of toxicity to the embryo or seed. The most useful gel for the encapsulation of somatic embryos was sodium alginate, a common food thickener made from sea algae. Artificial seeds are currently produced by mixing somatic embryos with 2% sodium alginate. The alginate is then dropped into a solution of calcium nitrate to complex the drops of alginate. After approximately 20 minutes, a rigid bead of complexed alginate is formed around the somatic embryo. The alginate bead provides a protective barrier for the fragile embryo while also facilitating the handling of the small embryos.

In addition to the encapsulation process, a hydrophobic membrane coating can be formed over the capsule. This membrane coating makes the capsules less tacky and increases the flowability. Capsule flowability is important for interfacing with automated seeding machinery where precision placement of the encapsulated embryo is required, such as seeding flats for transplant production. Initial seeding experiments using unfilled capsules (no embryos in the capsules) have been successfully singulated and placed into transplant flats using a greenhouse vacuum planter.

Planting and Conversion of Artificial Seeds to Plants

There has been little research done on the conversion of somatic embryos or artificial seeds to plants in a transplant-like soil environment. Most research has been on the process of forming somatic embryos and identifying gels or matrices that can deliver the somatic embryos. Very little research has focused on what to do with the somatic embryos beyond this point.

The germination of artificial seeds in soil, whether in a controlled environment such as an incubator, or a less controlled environment as exists under greenhouse and field conditions, requires the somatic embryos to be hardy and self-sufficient in order for root, shoot, and leaf formation to occur. Embryos must either store their own nutrients for growth and development or be supplied with an exogenous nutrient supply that functions as an "artificial endosperm." Our main research emphasis in the past two years has been to direct research and development towards prototype production and, thus, remove artificial seeds from the sterile, in vitro environment on which most research thus far has focused.

At Plant Genetics, Inc., we have emphasized efforts to obtain high conversion of artificial seeds to plants in a non-sterile soil environment. This shift in focus had initially required determination of planting methods and cultural practices which were amenable to artificial seed survival in soil. Current horticultural practices for true seed germination, such as soil types and watering systems, could be directly applicable for artificial seed germination, but the specific cultural techniques had to be determined for each crop. From

investigations using hydrated alfalfa somatic embryos, excessive overhead watering (misting) was shown to be detrimental to naked (non-coated) or encapsulated somatic embryos planted in the greenhouse. It appeared that the constant presence of water on the somatic embryo promoted extreme embryo browning and rotting. On the other hand, dry soil conditions were extremely detrimental to embryo survival, causing embryo desiccation and death. Therefore, it appeared that only a narrow range of soil moisture could be tolerated by the embryos and, as a consequence, the watering conditions needed to be tightly regulated. This differed from true seed as many seeds can survive dry soil conditions prior to radicle or shoot emergence. We have also begun to investigate the use of foggers to reduce the drying on the soil surface and maintain an even soil moisture level around the embryos.

Another focus was to improve embryo quality by directing our efforts on assessing whole plant formation in soil. The conversion of alfalfa somatic embryos was found to be much lower in soil compared with in vitro conditions. We typically obtained 10-30% conversion in soil while in vitro conditions yielded 70-90% conversion using high quality embryos. This indicated a lack of embryo vigor under soil germination conditions which may have indicated a requirement for nutrients such as those in the in vitro environment. The decline in conversion also demonstrated how different the soil germination environment was compared to in vitro conditions. Our goal for soil planting has been to achieve the same conversion rate in soil as in vitro. Along with low somatic embryo conversion in soil, plant stand and growth rates were also uneven, indicating variability in embryo vigor. We have recently been able to achieve 20-40% stand establishment of alfalfa plantlets from artificial seeds planted in the greenhouse by directing efforts to identify greenhouse growing conditions important for embryo survival as well as identify in vitro treatments which harden the embryo to withstand soil planting.

Important Hurdles for the Commercialization of Artificial Seed

The lack of a desirable genotype will probably be most limiting to artificial seed commercialization. This is due to a limitation on the understanding of the somatic embryogenesis systems. For many high-value genotypes, good somatic embryogenesis systems simply do not exist. Also, while advances are being made toward decreasing the cost of the somatic embryogenesis process, it appears that artificial seeds will have a difficult time competing economically with inexpensive true seeds and, thus, initially be used exclusively for high-value crops. Automation of the tissue culture process for embryo production may, therefore, be an important factor in reducing the cost of the labor-intensive production steps.

In addition, there are many other issues such as artificial seed storage, transport, and handling that have not been addressed.

All of these issues will be important in the overall efficiency of the artificial seed system.

Conclusion

Artificial seeds hold the promise of being a revolutionary propagation system in agriculture. While there are many hurdles to overcome before the system can be commercialized, much progress has been made in the past few years toward an initial greenhouse propagation method. Field use of artificial seeds is still further away, but research focus on the understanding of plant biology coupled with optimization of delivery and planting methods will push the artificial seed system closer towards commercialization.

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