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Review Paper

Production and Applications of Artificial seeds: A Review

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Abstract

Artificial seeds are most commonly described as encapsulated somatic embryos. They are product of somatic cells, so can be used for large scale clonal propagation. Apart from somatic embryos, other explants such as shoot tips, axillary buds have also been used in preparation of artificial seeds. Artificial seeds have a variety of applications in plant biotechnology such as large scale clonal propagation, germplasm conservation, breeding of plants in which propagation trough normal seeds is not possible, genetic uniformity, easy storage and transportation etc. For some plants such as ornamental plants, propagation trough somatic embryogenesis and artificial seeds is the only way out. In the present paper- the types, advantages, production methods and various applications of artificial seeds have been reviewed.

Keywords: Artificial seed, somatic embryogenesis, clonal propagation.

Introduction

The seed (or zygotic seed) is the vehicle that connects one generation to another in much of the plant kingdom. By means of seed, plants are able to transmit their genetic constitution in generations and therefore seeds are the most appropriate means of propagation, storage and dispersal[1]. Artificial seeds have great potential for large scale production of plants at low cost as an alternative to true seeds[2]. An artificial seed is often described as a novel analogue to true seed consisting of a somatic embryo surrounded by an artificial coat which is at most equivalent to an immature zygotic embryo, possibly at post-heart stage or early cotyledonary stage[3]. There are various advantages of artificial seeds such as; better and clonal plants could be propagated similar to seeds; preservation of rare plant species extending biodiversity could be realized; and more consistent and synchronized harvesting of important agricultural crops would become a reality, among many other possibilities[4]. In addition; ease of handling, potential long-term storage and low cost of production and subsequent propagation are other benefits[3].

The artificial seed production technique was first used in clonal propagation to cultivate somatic embryos placed into an artificial endosperm and constrained by an artificial seed coat. Today artificial seeds represent capsules with a gel envelope, which contain not only somatic embryos but also axillary and apical buds or stem and root segments[5]. Explants such as shoot tips, axillary buds and somatic embryos are encapsulated in cryoprotectant material like hydrogel, alginate gel, ethylene glycol, dimethylsulfoxide (DMSO) and others that can be developed into a plant. The coating protects the explants from mechanical damage during handling and allows germination and conversion to occur without inducing undesirable variations. They behave like true seeds and sprout into seedlings under suitable conditions[6].

However, as described in 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[7].

The need for artificial seed

A seed is basically zygotic embryo with enhanced nutritive tissues and covered by several protective layers. Seeds are desiccation tolerant, durable and quiescent due to protective coat. Such properties of seeds are also used for germplasm preservation in seed repositories. Zygotic embryo seeds are the result of sexual reproduction that means 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. Primary problem associated with such seeds is, on one hand for many crops, such as fruits, nuts, and certain ornamental plants; it is not possible to produce a true-breeding seed from two parents due to genetic barriers to selfing. On the other hand many crops, such as forest trees, the generation time is too long to achieve rationally an inbred breeding program. This is the major disadvantage of zygotic seeds. Therefore, for such crops, propagation is accomplished either vegetatively by cuttings or the use of relatively low-quality open pollinated seed is tolerated.

After the discovery of somatic embryogenesis in 1950 it was possible to have an alternative of conventional zygotic seeds. Somatic embryo arises from the somatic cells of a single parent. They differ from zygotic embryos since somatic embryos are produced through *in vitro* culture, without nutritive and protective seed coats and do not typically become quiescent. Somatic embryos are structurally equivalent to zygotic embryos, but are true clones, since they arise from the somatic cells of a single parent. The structural complexity of artificial seeds 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 non quiescent, depending on its usage. 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.

Types of artificial seeds

There are various types of artificial seeds; first two are essentially uncoated somatic embryos; i. uncoated non quiescent somatic embryos, which could be used to produce those crops that are now laboriously micro propagated by tissue culture; ii. uncoated, quiescent somatic embryos would be useful for germplasm storage since they can be hand-stored in existing seed storage repositories. The other categories are; iii. non quiescent somatic embryos 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, celery, seedless watermelon, and other vegetables and iv. 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[8]. Many studies have been conducted on synthetic seed production in horticultural crops but the efforts in field grown crops are limited. So, there is a greater scope for synthetic seeds in commercial crops and ornamental plants[9].

Advantages of artificial seeds

There are various advantages of artificial seeds. One of the chief advantages is the possibility of large scale propagation and mixed genotype plantations – very much suitable for large scale monoculture. Another big advantage is the germplasm conservation of elite and endangered or extinct plant species. Other advantages are easy handling during storage; transportation and planting and inexpensive transport reason being their small size; storage life comparable to natural seeds; product uniformity – as somatic embryos used are genetically identical. In addition, other potential benefits can be direct field use, study of seed coat formation, fusion of endosperm in embryo development and seed germination; for production of hybrids in plants with unstable genotypes or show seed sterility. It can be used in combination with embryo rescue technique[3,7].

Procedure for the Production of Artificial Seeds

The general procedure of artificial seed production is described in figure 1. There could be a number of possible artificial seed systems, depending upon the type of artificial seed produced, need of artificial seeds, the economic feasibility and it will vary greatly among species[7]. The development of the ideal viable, quiescent, low-cost artificial seed has been described as a 10-step process[10]. First of the steps is the selection of the crop based on technological and commercial potential followed by the establishment of a somatic embryo system (species-specific). Next is the optimization of the clonal production system (optimizing protocols to synchronize and maximize the development of normal mature embryos capable of conversion to normal plants. Automation of embryo production is followed by this. After that, post-treatment of mature embryos to induce quiescence, development of an encapsulation and coating system, optimization and automation of the encapsulation system and conversion requirements for greenhouse and field growth (watering, fertilizer, transplantation, etc.) are followed. Identification and control of any pest and disease problems that may be unique to artificial seeds and 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) are last steps[10]. 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[7].

Components of the artificial matrix: The synthetic endosperm or artificial matrix is composed of minerals and vitamins from the MS culture medium[11] supplemented by 0.5 mg L-1 of indolacetic acid (IAA), 0.5 mg L-1 of naphthalene acetic acid (NAA), 2 mg L-1 of 6-benzyl aminopurine (BA), 2 mg L-1 of Fe-EDTA and 30 g L-1 of sucrose. Depending on the encapsulation treatment applied, agar is replaced by sodium alginate at 4%, 3% and 2%. The complexing agent of the capsules is applied through immersion in calcium chloride ($CaCl_2$) at different concentrations and determined time intervals. Finally, these are washed in sterile water for 40 min[12].

Encapsulation of somatic embryos: The somatic embryos isolated are submerged in a solution of sodium alginate, according to the type of encapsulation applied, and subsequently suctioned through a micropipette to provide a protective capsule. In order to seal the capsules, they are then submerged in a complexing solution of $CaCl_2$ for a determined period of time followed by washing in sterile water for 40 min. This process is carried out under aseptic conditions in a laminar flow chamber, laminar with prior sterilization of the material and culture medium. Finally, the artificial seeds are cultivated in a germination medium in Petri dishes with macro and micronutrient from the MS medium supplemented with 30 g/l of sucrose and 7 g/l of agar-agar. They are then left in the culture chamber at a temperature of 25ºC in complete darkness[7].

Applications of Artificial seeds

Artificial seeds have vast application in different fields of plant biotechnology for cultivation of various plant species. They offer the opportunity to store genetically heterozygous plants or plants with a single outstanding combination of genes that could not be maintained by conventional methods of seed production due to genetic recombination exists in every generation for seed multiplication[13]. In this section, key applications of artificial seeds technology in various fields have been discussed.

Many species are sterile and produce no seeds. Somatic embryogenesis is an alternative with respect to the cuttings to propagate these plants. Other species, including some tropical produce recalcitrant seeds that can not be dried. Consequently, long-term storage in gene banks in these species is not possible. The artificial seeds can be an alternative as more is learned about the mechanism by which this type of seed has no tolerance to desiccation[14]. In autogamous species, where the production of hybrid seed is difficult and expensive, the artificial seed technology offers many advantages and opportunities.

One of the limitations of the method of micro propagation is that they should be in the same physical site of tissue culture laboratories and greenhouses, as production of propagules must be synchronized in periods of peak demand in the market. Artificial seed production in these species would not link the laboratory facilities of the greenhouses[15].

The market for ornamental plants is growing every year. The high cost of production of these species is given by the diligence of the micro propagation and manpower needed in the later stages of propagation and production. The use of somatic embryogenesis system in these species would significantly reduce labor costs[16].

Coniferous forest species can be propagated cheaply through seeds. The conventional breeding programs in these species are very time consuming because the life cycle of conifers is very long. Coniferous forests are very heterogeneous and that the seed of outstanding individuals will not necessarily give rise to improved offspring. Artificial seed has the ability to clone those overhanging trees at reasonable cost and in minimum time[17].

In the commercial sector, it is very difficult to produce low-cost hybrid seed species such as cotton (Gossypium hirsitum L.) and soybean (Glycine max Merril.) because they have cleistogamous flowers and abscission problems as the seed that is currently used comes from self-pollinating species. However, hybrid seed is produced in small quantities in a very laborious by hand pollination. This small volume of hybrid seed could be massively increased through artificial seed technology. Thus, the hybrid force would be used commercially to originate a significant reduction in costs[18].

In certain vegetable species, used hybrid seeds are expensive and therefore the plant value is very high. For example, tomatoes and seedless watermelon hybrid seeds are used in very high cost. The reason for this high cost is that pollination is done by hand, requiring intensive labor. In other species, vegetative reproduction is used it also consumes much time, space and labor. The use of artificial seed technology can significantly reduce costs by reducing the labor required, time and space in case of these plants[16].

Sowing seed of synthetic varieties is a common practice in forage species such as alfalfa (Medicago sativa L.) and orchargrass (Dactylis glomerata L.). Such varieties from selection and crossing of lines are phenotypically uniform but different genotypes. These lines to cross freely year after year to produce seeds, heterozygous and heterogeneous populations originate. The use of artificial seed allows multiplication of outstanding genotypes and genetically uniform, since this method does not require that annually cross-pollination is carried out to produce plants[19].

The vast majority of fruit species are propagated by vegetative means because of the presence of self-incompatibility and breeding cycles very long. The use of synthetic seed facilitates its spread. However, the most useful artificial seed would be in the conservation of germplasm of these species[20]. Currently seed banks are maintained as live plants in the field. This method of conservation is very expensive and dangerous, as it is exposed to natural disasters. The use of artificial seeds would retain these clones in a small space, under controlled conditions (cryopreservation) and without the danger of natural disasters. In addition, this system of germplasm conservation would be particularly useful in tropical species where conservation means are inadequate or nonexistent. The vine (Vitis spp.) is a practical example of this system of conservation.

In cross-pollinated species like maize, where production of hybrid seed is a widespread practice. The creation of hybrids through a conventional breeding program consumes much time and resources in obtaining and maintaining appropriate parental lines. One possibility is the use of artificial seed to propagate outstanding genotypes without the need to generate parental lines costly in time and money. This could facilitate the commercialization of new hybrids and encourage the emergence of new seed companies, as it would be possible to produce new hybrids without the need for large amounts of parental lines[17].

In autogamous species such as wheat, barley and oats where hybrid seed production at commercial level is not possible by high production costs, artificial seeds would spread the hybrid seed. In this case, produce small quantities of hybrid seed by hand and then with the technology of artificial seed multiplication would be carried out mass[21].

There are a growing number of species that are in the process of extinction. Indiscriminate felling of forests, increasing desertification, disappearing forests, etc. increases the changes of extinction of species. Many of these native species cannot be propagated vegetatively, or produce very low quantities of seed. For this reason the artificial seed is an alternative for these species. For example, in Australia, eucalyptus tolerant to saline soils has been obtained. These eucalypts cannot multiply by cuttings or by seed true. One option is the artificial seed technology[21].

Crops from genetically modified plants have boomed in recent years. There is little information about what happens to these GMOs in the process of sexual reproduction. It is possible that during sexual multiplication, the introduced genes from other species are meiotically unstable and lost. With the use of artificial seed technology would avoid such risks. Similarly, this technology could be used in the propagation of somatic hybrids and cytoplasmic (obtained through protoplast fusion) and in sterile and unstable genotypes[21].

Hwang et al. attempted propagation of perennial brown alga *Sargassum fulvellum* through somatic embryogenesis and artificial seeds. *Sargassum fulvellum* is a brown alga introduced to the seaweed cultivation industry in Korea. According to the authors, this species offers good potential to diversify seaweed cultivation in Korea[22]. Similarly, there is another brown algae *Undariopsis peterseniana,* which is an endangered annual brown alga in Udo, Jeju Island, Korea. There is current interest in the commercial-scale aquaculture of this species for warm-water species development in Korea. Hwang et al. investigated growth and maturation of this alga by sometic embryogenesis and artificial seeds[23].

Artificial seeds of *Dendrocalamus strictus,* commonly called the male bamboo, were produced by encapsulating somatic embryos that had been obtained on MS medium containing 3.0 mg 1 -I 2,4- dichlorophenoxyacetic acid (2,4-D) and 0.5 mg I -I kinetin (Kin), in calcium alginate beads. A germination frequency of 96% and 45% was achieved in vitro and in soil, respectively. The in vivo plantlet conversion frequency was increased to 56% following an additional coating of mineral oil on the alginate beads. They were able to achieve the germination of artificial seeds into plantlets[24].

Conclusion

Artificial seeds have wide spread applicability in large scale plant propagation. For some ornamental and extinct plant species, it is the only means of propagation. Apart from this, they have been used in commercial production of autogamous plant species, genetically modified plants, conifers, algae etc. In sum, artificial seed technology has influenced almost every aspect of plant biotechnology and has the potential to become the most promising and viable technology for large scale production of plants.

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Single parent

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Callus => Globular => Heart/Torpedo Stage => Somatic Embryo
(Somatic Embryogenesis)

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Mature somatic embryo

↓

Synchronize and singulate somatic embryos

↓

Mass production of somatic embryos

↓

Standardization of encapsulation

↓

Standardization of artificial endosperm

↓

Mass production of synthetic / artificial seeds

↓

Greenhouse and field planting

Figure-1
Procedure for production of artificial seed