

ARTIFICIAL SEEDS

Introduction and Definition

The demand for artificial seed technology started after the discovery of somatic embryo production in various plant species in vitro. Artificial seeds, which are also known by other names such as “synseeds”. It is “an encapsulated single somatic embryo”. An artificial seed was later defined “a somatic embryo that is engineered for the practical use in commercial plant production”. The concept of artificial seeds was then limited to those plant species in which the production of their somatic embryos could be demonstrated.

The definition of artificial seeds depends on the similarity in physiology, morphology, and biochemistry of somatic embryos to zygotic embryos. Considering the recalcitrance to somatic embryogenesis in some plant species, the concept of artificial seeds was later extended to be the encapsulation of a range of in vitro-derived propagules. The definition of artificial seeds was then extended to be artificially coated somatic embryos (usually) or other vegetative parts such as shoot buds, cell aggregates, auxiliary buds, or any other micropropagules, provided that they have the capacity to be sown as a seed and converted into a plant under in vitro or ex vitro conditions. They should also be able to keep this ability for an extended period (storage ability). Therefore, artificial seeds can eliminate the acclimation steps necessary in micropropagation and give breeders greater flexibility. Various plant materials have since been used for artificial seed production including somatic embryos, shoot tips, axillary buds, nodal segments, protocorm-like bodies (PLBs), microshoots, and embryogenic calluses. Several studies have investigated the production of artificial seeds working with different plant species, including vegetables, fruits, medical plants, ornamentals, forest trees, orchids, and cereals.

Although the vast majority of artificial seeds are produced from encapsulated in vitro-derived propagules, the possibility of encapsulating in vivo-derived propagules have been confirmed in some plant species. For example, the success of encapsulation of dormant vegetative buds of an in vivo-cultured mature mulberry tree. Furthermore, the production of artificial seeds from encapsulated *Curcuma amada* microshoots.

The Importance, Uses and Advantages of Artificial Seeds

By using the benefits of a vegetative regeneration system with the capability of long-term storage, different applications of artificial seeds in agriculture have been made. Crops which are used for artificial seed production can be classified into two categories:

- Those that have a high quality of somatic embryos, and
- Those with a strong commercial basis.
 1. Artificial seeds could be a good tool to propagate these types of plants and to store their propagules for a reasonable period of time.

2. Artificial seed production is an essential technique for the proliferation of plant species which are not able to produce seed, such as seedless grapes and seedless watermelon.
3. Artificial seeds can be employed for production of polyploids with elite traits, avoiding the genetic recombination when these plants are propagated using conventional plant breeding systems, thus saving on time and costs (Fig. 18).
4. Artificial seeds can be also used in the proliferation of male or female sterile plants for hybrid seed production.
5. Artificial seed production through the use of somatic embryos is an important technique for transgenic plants, where a single gene can be placed in a somatic cell and then this gene will be located in all the plants produced from this cell.
6. Therefore, artificial seeds could be an efficient technology used for reproduction of transgenic plants.
7. The encapsulation technology can be considered as a promising approach that can be used for the exchange of plant materials between public and private plant tissue culture laboratories, and also to achieve germplasm conservation and the propagules that are derived from in vitro or by micropropagation applied directly in nurseries or in a field.
8. Artificial seeds, which are produced using tissue culture techniques that are aseptic, are free of pathogens, giving great advantages to these materials for transport across frontiers and for avoiding the spread of plant diseases.
9. Artificial seeds are also valuable in terms of their role in providing protective coating, increasing the level of micropropagule success in the field. These micropropagules need a protective coating to increase successful establishment in the field situation because of the sensitivity of uncovered micropropagules to drought and pathogens under natural environmental conditions.
10. Artificial seeds are more durable for handling, transportation, and storage.
11. Artificial seed production is also a useful technique as a clonal propagation system in terms of preservation of the genetic uniformity of plants, straight delivery to the field, low cost, and fast reproduction of plants (Fig. 18).
12. Artificial seed production may offer a tool suitable for the extensive scale-up required for multi-clone commercial production.
13. Moreover, the use of this technique economizes upon the space, medium, and time requested by the traditional tissue culture methods.
14. Artificial seed production has great advantages in comparison with traditional tissue culture methods.
15. Artificial seeds are reasonably inexpensive to produce and easy to handle, plant, and transport. They can also be stored for a long period using dehydration and cryopreservation techniques.
16. Artificial seeds can be very useful for grass species as well as many others.

17. Artificial seeds (encapsulated somatic embryos, in specific) can open new vistas for land restoration and the rehabilitation of wild lands (rangelands, grasslands, forests, abandoned mine lands, etc.) affected by
18. overgrazing or climate change. Unfortunately, because of the abovementioned problems, the seed bank in the soil and the natural seed production of the mother plants cannot recover the loss of naturally reserved seeds year after year of pressure. Therefore, mass production of embryos or embryogenic calluses and their use for artificial seed production are important for the future of land restoration.
19. However, there is a limited number of studies and literature that investigate the potential use of artificial seeds for land restoration, and this could be an important point for future research. The use of tissue culture and micropropagation techniques with several plant species were summarized.
20. However, more investigations are required to find out the possibility to develop the micropropagation systems to produce artificial seeds.
21. restoration. However, there is a limited number of studies and literature that investigate the potential use of artificial seeds for land restoration, and this could be an important point for future research.

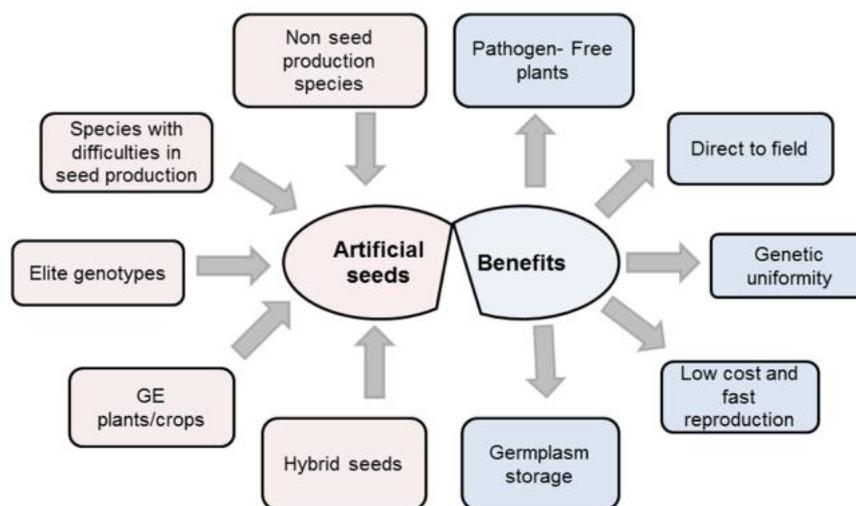


Figure 18. Artificial seed uses and benefits.

Artificial Seed Concept

The artificial seed structure mimics that of the conventional seed. It consists of both explant material, which imitates the zygotic embryo in the conventional seed, and the capsule (gel agent and additional materials such as: nutrients, growth regulators, anti-pathogens, bio-controllers, and bio fertilizers), which emulates the endosperm in the conventional seed.

Essential Requirements for the Production of Artificial Seeds

1. *Explant Material*

Explant materials are the basic generative component of the artificial seed. It could be *Somatic Embryos*. Somatic embryos are the most common micropropagule used for artificial seed production because their structures are able to produce the radical and plumule axis, which has the capability to progress into the root and shoot in a single step. Artificial seeds produced through somatic embryos can also provide high levels of reproduction. Plant lines, which are produced via somatic embryos, are capable of keeping their regenerative capacity for a long time, resulting in uniform plant production, because it avoids the dedifferentiation callus stage, and consistent genetic structure production.

The use of somatic embryos for artificial seed production has become widespread over time, and the number of species which seem to have propagation ability using this technique is increasing. The production of artificial seeds via somatic embryos have been investigated in several plant species.

On the other hand, some authors consider that the degree of vigour or maturity of the embryos at the moment of being encapsulated can influence the germination of encapsulated somatic embryos (ESEs). It was also suggested that encapsulation can affect embryo respiration and this in turn might influence the germination and viability of somatic embryos. However, low germination and conversion rates were reported with different woody species mainly due to deficiencies and asynchronous maturation of the embryonic pole, which led to difficulties in the final stages of the process.

Separation of somatic embryos

Ultrasound is used to separate clusters of somatic embryos, which has been used successfully in separating embryos of carrots, *Quercus uber*, grapes, and cherries. Special devices have been designed for this, as they work efficiently. For carrot embryos, the device is calibrated to operate at 190 KHz and with 0.5-1.5 watt oscillations. For the embryos of large trees, it is 175 kHz and oscillations of 0.7 watts. The embryos are separated based on size after smoothly turning on the ultrasound device, as the particles are much smaller than the sound wavelengths used. Because the length of the bodily embryos is about 1 mm, while the length of the sound wave is about 7 mm, which holds the embryos in the sound antinodes of the sound speed range, where the sound energy is at its lowest, and the embryos are not damaged as a result of the force of sound. Small embryos and undifferentiated cells are not held sufficiently in flow-based devices and sieves to be sorted aside. Ultrasonic somatic embryo sorting can replace screening techniques, which rely on sorting embryos by size. Still, sound waves are less harmful and more efficient and are currently used in fermenting animal cells.

Encapsulation of somatic embryos

Many materials, such as acarose, sodium lignan, and polyoxyethylene, were used to encapsulate somatic embryos. Eight materials were tested for encapsulating citrus embryos, and it was found that polyoxyethylene was the best in its ability to form an enveloping layer for the bodily embryo. This substance has easy solubility in water and

has no adverse effect on embryo germination. The same material was used successfully in encapsulating celery plant embryos. Numerous studies have been devoted to producing wet, encapsulated somatic embryos of celery and jet plants using wet gel, especially sodium lignite. The research results have been marketed in the precise propagation of these two plants, and their low cost has been proven to produce high-quality, field-homogeneous plants with genetic and phenotypic characteristics. Artificial seeds have been produced commercially for many plant species. We mention, but are not limited to, garlic, rape, cauliflower, carrots, cotton, lettuce, jet, rice, yellow corn, and many grasses, forest trees, shrubs, fruit trees, vegetable crops, and ornamental plants.

Research has proven the suitability of water-soluble gelatin for somatic embryo encapsulation and artificial seed production. Two methods were used to encapsulate the embryos. The first involves encapsulation using gelatin complexes using the dropping method. Then, Molding, for example, but not limited to, the somatic embryos of the jet plant are mixed with 2% w/v sodium lignite and then dropped into a solution of calcium nitrate (100 mM). The drops freeze completely within 30 seconds to become surrounding the embryos. Wet covers were and still are necessary for the embryos. When their humidity is reduced, they are affected by any poor tolerance to reduced humidity due to a decrease in their transformation rate into poor conversion plants. It has generally been observed that somatic embryos coated with Hydrogel have conversion rates approximately equal to uncoated embryos when grown in laboratory conditions. Examples of reducing the moisture of somatic embryos of a crop, jet, are: It was found that the loss of moisture for 5 days at a temperature of 20°C produced somatic embryos that were able to transform into plants at a rate of 3%, while the embryos coated with sodium alginate produced a transformation rate of 62%. It was possible to increase the final transformation rate by adding charcoal to the gel (perhaps), causing increased embryo breathing in the medium. Other examples include the somatic embryos derived from the microspores of the barley plant, which were coated with a moist gel of sodium lignite to become artificial seeds, in which a germination rate reached 80% when compared to spores. Unwrapped flour, whose germination rate will not exceed 62%. On the other hand, coated embryos can be stored for 6 months, while the storage period for naked embryos will not exceed 3 weeks, and delaying beyond that period leads to their destruction. Specialized companies have developed machines to coat bodily embryos with materials suitable for the embryo's growth when they are seeded, so these artificial seeds are ready for mechanical seeding.

In principle, it isn't easy to evaluate the encapsulation process due to the quality of the encapsulated embryo. It may encapsulate already poorly developed embryos, which may lead to non-occurrence of germination or its delay, in addition to selecting the embryo at the incorrect stage.

However, it produced artificial seeds that germinated naturally, forming a bipolar root, a vegetative growth axis, and a cotyledon. However, as was previously mentioned, germination and embryo development may not be convincing. This is why many laboratories have devoted their efforts to producing mature embryos identical to the mother plant and of high quality, thus producing high-quality artificial seeds in

commercial quantities. The goal was achieved after the criterion became the embryo's development into a complete plant in a process called Conversion.

Figure 19 illustrate the structural differences between artificial and natural seeds after making cross-sections and longitudinal sections of both of them, as the similarities between the two are highlighted, except for surrounding the physical embryo with synthetic nutrients that provide it with nutrients until it completes its germination so that it can become self-sufficient after it becomes a complete plant that performs the process of photosynthesis.

Coatings of nutrients, pesticides and inert materials were added to make the seed size suitable for automatic seeding (the form of synthetic seeds on a commercial scale and under different names, including seed bombs).

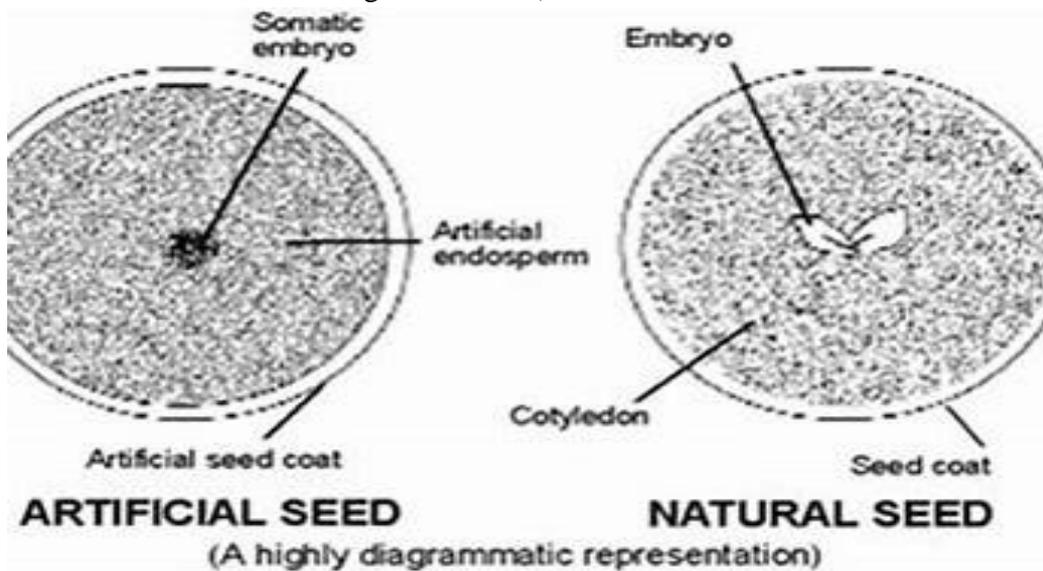


Figure 19 illustrate the structural differences between artificial and natural seeds.

Uncoated desiccated synthetic seeds

Great efforts have been made to develop artificial seeds with low moisture content after it was previously believed that drying or reducing the moisture content of bodily embryos might lead to their damage, with very low rates of transformation into plants. The first success in improving the transition from somatic embryos to plants was achieved after lowering the initial humidity in grape plants, and this was encouraging for applying the technology to somatic embryos of other plants.

When the moisture content of the embryos of the jet crop was reduced, for example, to 8-15%, they retained their vitality after being stored for 12 months under conditions of relative humidity and room temperature. With scientific progress, it has become clear that physical embryos dried to reasonable humidity levels are more viable than their non-dried counterparts, have a longer lifespan and remain viable. The length of storage period for synthetic seeds is less than that of natural seeds.

Types of artificial seeds

Two types of artificial seeds (encapsulated somatic embryos) are commonly produced: desiccated and hydrated.

1. Desiccated artificial seeds

Desiccated artificial seeds are achieved from somatic embryos either naked or encapsulated in polyoxyethylene glycol followed by their desiccation. Desiccation can be applied either rapidly by leaving artificial seeds in unsealed petri dishes on the bench overnight to dry, or slowly over a more controlled period of reducing relative humidity. These types of artificial seeds can be only made in plants whose somatic embryos are desiccation-tolerant.

The desiccation tolerance of somatic embryos can be induced using a high osmotic potential of the maturation medium. The osmotic potential could be increased by using a high gel strength or by the addition of permeating osmoticants such as mannitol, sucrose, etc.. Desiccation can also be induced by applying sub-lethal stresses such as nutrient deprivation or low temperature, since these treatments have been reported to have similar effects on desiccation tolerance.

2. Hydrated artificial seeds

Hydrated artificial seeds can be produced by encapsulating somatic embryos in hydrogel capsules. They are produced in plant species which are recalcitrant and sensitive to desiccation. Encapsulation has been expected to be the best method to supply protection and to convert the in vitro micropropagules into 'artificial seeds' or 'synseeds', and it is an important application of micropropagation to develop the success of in vitro-derived plant delivery to the field. However, somatic embryos need to be encapsulated in a suitable material that promotes germination.

Apical shoot tips and axillary shoot buds and microshoots

Although unipolar axillary shoot buds and apical shoot tips contain no root meristem, they have been encapsulated to produce artificial seeds in several plant species. However, although these explants required some special treatment for induction of the reformation of roots before the encapsulation stage, some studies reported the conversion of encapsulated buds of banana and mulberry into plantlets without specific induction treatment. Ganapathi mentioned that 100% conversion of encapsulated banana shoot tips into plantlets was obtained using White's culture medium, and these plantlets were effectively based in soil. Piccioni and Standardi reported that encapsulated micropropagated buds of six woody species—apple (*Malus* spp.), blackberry (*Rubus* spp.), birch (*Betula pendula*), kiwifruit (*Actinidia deliciosa*), raspberry (*Rubus idaeus* L.), and hawthorn (*Crataegus oxyacantha*)—were successfully regrown after encapsulation and cultivation on enriched media. Working in M.26 apple rootstock, encapsulated apical buds (artificial seed) showed higher levels of conversion in comparison with artificial seeds from axillary buds (the maximum conversion rates for encapsulated apical and axillary buds were 85% and 25%, respectively). Lata mentioned that 100% conversion of encapsulated axillary buds was produced in the suitable capsulation matrix and the plantlets produced were effectively passed to the soil. These consequences prove the ability of such explants for encapsulation and artificial seed production. The encapsulation of cauliflower microshoots for artificial seed production was successfully achieved and the capacity to convert in commercial substrates was confirmed as well.

Other explant materials

Several other explant materials, such as embryogenic masses and protocrom-like bodies, have been investigated to test their ability to produce artificial seeds, although the supporting of embryogenic masses in culture tubs is expensive and labour-intensive, and mechanically provoked bio-reactors require regular transfer of tissue to new media. Nonetheless, Onay were able to successfully produce artificial seeds via an embryogenic mass. They reported that the encapsulated embryogenic mass fractions regained their primary reproductive ability after two-month storage. The production of artificial seeds through encapsulated protocrom-like bodies of orchid (*Geodorum densiflorium*) was investigated. These authors mentioned that the encapsulated protocrom-like bodies retained their viability after three-month storage at 4 °C, while non-encapsulated protocrom-like bodies appeared non-viable after one-month storage at 4 °C. However, there is a variety of explants that could be used for artificial seed production, and this depends essentially on the plant species.

Artificial Seed Gelling Agents and Adjuvant Materials

The basic limitation for using somatic embryos as micropropagules for plant propagation is that the somatic embryos are delicate structures without a quiescent resting stage. Therefore, they require essential supplementary tissues that should provide the nutrient elements and a protective layer, which makes them easier to handle and store. Thus, the main objective of artificial seed research is the production of an artificial seed structure that stimulates the conventional seed in its characteristics (such as handling, storage, viability, and germination level).

At the beginning of artificial seed studies, polyoxyethylene was selected as a suitable capsulation material to encapsulate celery embryos due to their positive properties, such as sustained embryo growth, non-toxicity to explants, and solubility in water. Later applied to alfalfa embryos, Redenbaugh reported a new technology using hydrogel encapsulation. Since then, the hydrogel materials have been the main structure for somatic embryo encapsulation.

However, although many gel materials such as agar, alginate, carrageenan, guar gum, and sodium pectrate were investigated for artificial seed production, alginate matrix was discovered to be the optimal encapsulation for artificial seed production because of its sensible thickness, weak spinnability of solution, low toxicity of micro-organism, low expense, bio-suitability characteristics and, fast gellation. This material improves capsule structure and bead rigidity, supplying better protection to covered explants against mechanical hurt. The major principle for alginate encapsulation formation depends on the exchange ions between Na^+ in sodium alginate with Ca^{2+} in $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, which happens when sodium alginate droplets involving the artificial embryos or any other plant propagule is dropped into the $\text{Ca}^{2+} \cdot 2\text{H}_2\text{O}$ solution, producing stable explant beads. The solidity and rigidity of the capsule (explant beads) depends upon the two gelling agents' (sodium alginate and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) concentrations and mixing duration. Nutrients

should also be added to the artificial endosperm in order to maintain germplasm survival, obtain faster explant growth, and supply the energy required for germination, which is normally provided by endosperm or gametophyte tissue in true seeds. On other hand, the addition of growth regulators and nutrients to the capsule is an essential factor to a successful artificial seed production technique, increasing the competence of germination and viability of these seeds. These materials are considered as artificial endosperm and they also play an important role in the artificial seed storage capability. However, there are many other materials such as pesticide, antibiotics, and fungicide, which have positive effects in the capsule features.

Artificial Endosperm Structure and Their Effects in the Artificial Seed Characters

The endosperm of artificial seeds could be similar to the endosperm of seeds, but can also be manipulated so as to control growth and reduce the difficulties of the germination of somatic embryos. While Saiprasad mentioned that usually 3% sodium with complexing solution containing 75 mM $\text{Ca}_2\text{Cl}_2 \cdot 2\text{H}_2\text{O}$ for half an hour mixing duration provides the optimal structure for artificial seed bead formation, Ara indicated that generally 2% sodium alginate gel upon complexation with 100 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ is the best. However, not just the concentrations of gel agents, but also the mixing duration has important effects on the rigidity and hardness of artificial seeds, which in turn greatly affect their characteristics (such as germination, storage ability). Daud mentioned that the germination level of African violet species was 72–80% when 30 min sodium alginate exposure duration was applied, compared to 52% when 10 min exposure duration was applied. However, these factors depend on others such as plant species, explants type, and the aim of the study, such as for short storage, long storage, or to obtain high germination level. However, lots of studies working in different plant species have investigated the optimal capsule structure which provides the highest germination rate, best seed viability, and their effects in the artificial seed storage duration.

The ultimate viability of the artificial seeds can be affected by the matrix material or simulated endosperm, as the matrix is responsible for the immediate surrounding of the plant materials. The temporal and quantitative supplement of growth regulators and nutrients along with an optimal physical environment can determine the quality of artificial seeds. An artificial seed can also be used as a carrier for micro-organisms, nutrients, antibiotics, plant growth regulators, pesticides, and fungicides. Also, it provides not only the physical protection for embryos but also the carbon source and growth regulators to control and sustain growth through germination. The endosperm of artificial seeds could be similar to the endosperm of seeds, but can also be manipulated so as to control growth and to reduce the difficulties of the germination of somatic embryos.

Artificial endosperm has a great effect on the germination level. In this way, Ara mentioned that the percentage of encapsulated somatic embryos germination of *Mangifera indica* L. was higher than non-encapsulated somatic embryos of the

similar size in the same medium. Many studies also have demonstrated the important role of artificial endosperm structure (capsulation structure) in the artificial seed storage capability. Lakshmana Rao and Singh indicated that the reduction in germination level of encapsulated somatic embryos of hybrid *Solanum-melongena* was much lower than in naked somatic embryos after 60-days storage at 4 °C. Furthermore, while the encapsulated pieces of embryos mass got back their basic proliferative ability after 60-days storage, naked fragments failed in that aspect.

7. Artificial Seeds Storage Ability

Several studies have investigated the artificial seed's storage capability. Rai reported that a high concentration of sucrose or ABA could be useful for short-term conservation of guava (*Psidium guajava* L.) because of their temporary inhibition in encapsulated somatic embryos germination. Working with *Rauvolfia serpentina* and applying three different temperature degrees (20 °C, 12 °C, and 4 °C), Ray and Bhattacharya indicated that 4 °C, where storage achieved up to 14 weeks with high regrowth percentage, was the optimal temperature for short storage duration. However, while short artificial seed storage can be obtained by applying several procedures—such as using suitable temperature (usually 4 °C), suitable capsulation materials, and optimal storage conditions (reduced heat, light, oxygen, etc.)—long storage can be achieved using dehydration and/or cryopreservation techniques.

Fabre and Dereuddre, working on *Solanum* shoot tips and aiming to increase the tolerance of plant tissue to dehydration–cryopreservation storage conditions, reported a full protocol for encapsulation-dehydration and storage. This protocol consists of three procedures: (a) preculturing encapsulated explants in a medium containing high concentrations of sucrose; (b) drying the encapsulated micro-organism; and (c) direct plunging into liquid nitrogen. Unfortunately, few research projects have investigated in depth the artificial seed preservation (dehydration-cryopreservation), and this technique still needs more studies in view of the great value of artificial seeds as an easy and cost-efficient method of germplasm preservation. Furthermore, artificial seed conservation facilitates the exchange and distribution of trait plant germplasm, decreasing the requirement for transferring and subculturing out of season.

Limitation of Artificial Seeds

The main requirement for an efficient artificial seed production protocol is the large-scale production of highly valuable micropogules suitable for encapsulation in sodium alginate matrix, at low cost per culture unit. However, although the design of such systems was achieved in some plant species, such as cauliflower (*Brassica oleracea* var. botrytis L.), the micropogulation system is still one of the major limitations of the development of artificial seed technology.

Although the use of somatic embryos has been widely reported for artificial seed production in various plant species, there are some major challenges that need to be solved to improve the efficiency of these protocols. The advantages of artificial

seed technology are encountered by challenges such as limitations in storage caused by lack of dormancy, synchronic deficiency in somatic embryo development, improper maturation, low levels of conversion into plantlets, limitation in production of viable mature somatic embryos, and the reduction of viability and plant recovery when the artificial seeds are stored at low temperature.

In the species that are recalcitrant to somatic embryogenesis, the possibility of using non-embryogenic propagules for artificial seed production was investigated in different plant species and reported to be a promising pathway as a propagation method. However, this pathway encountered some complications such as the difficulties of achieving one rooting step for non-embryogenic artificial seeds. The difficulties of sowing artificial seeds directly in soil or in commercial substrates such as compost, vermiculite, etc., under non-sterile conditions are considered to be one of the main limitations of the practical use of this technique.

Disadvantages of seed synthesis

Despite the great benefits of synthetic seeds and what will be achieved in the future in terms of increasing plant cover and diversity, reducing desertification, and reducing production costs, some technical limitations have emerged that can be overcome with the development of scientific research and mechanization. Below, we list the most important of these determinants:

1. There was a decrease in gaseous exchange in the encapsulated somatic embryos with a decrease in the rates of their transformation into plants with an increase in gel percentage. For example, a sharp decrease in oxygen level was recorded inside the calcium alginate gel, and 70% of the alginate balls were devoid of oxygen. The solution is to increase the oxygen content of the balls covering the embryos. Perfluorochemicals were used to be oxygen carriers after mixing them with the gel. Many polymers are still under testing, and the problem is hoped to be solved.
2. The need for experience, capital, and the establishment of on-site laboratories in countries and regions specialized in producing a particular crop, with the need to develop infrastructure such as roads, refrigerated warehouses, refrigerated means of transportation, continuous electrical energy sources, and others. This point is considered one of the most important challenges facing agricultural development in undeveloped countries.

Large-scale production of synthesis seeds

The need for synthetic seeds increases with the increase in population and agricultural area, to be millions or even billions of embryos of field and horticultural crops, which can only be produced through appropriate bioreactors. However, scientific evidence indicates an increase in the rate of transformation in solid media more than in liquid media (reactors). In large-scale production, there is an urgent need to develop packaging methods suitable for automated seeding and various types of embryos. There is a simplified method for producing somatic embryos, in which the somatic embryos move in a vibrating bowl. The embryos fall out one by one and are coated with sodium

alginate. The movement of the embryos, air, and gel formation are monitored through sensors attached to a device.

Conclusions

Artificial seeds were produced successfully from encapsulated plant propagules in different plant species. Procedures were optimized and proper plantlets were obtained. This technique has great advantages such as: a cost-effective delivery system, minimization of the cost of plantlets, simple methodology with high potential for mass production, a promising technique for the direct use of artificial seedlings *in vivo*, and a high storage capacity. The advances of this technique depend on the plant species in the first step.

However, despite the advantages of artificial seeds, further research is required in order to improve root formation of non-embryogenic artificial seeds. More investigations are needed to improve the capacity of artificial seed cultivation in commercial substrates and under non-sterilized conditions. This could be improved by the use of suitable types and concentrations of anti-diseases and antibiotics, and further detailed research is needed for improvement in the artificial seed cryopreservation capacity in some plant species.