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Review

Artificial Seeds (Principle, Aspects and Applications)

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Abstract: Artificial seeds are artificially encapsulated somatic embryos (usually) or other vegetative parts such as shoot buds, cell aggregates, auxiliary buds, or any other micropropagules which can be sown as a seed and converted into a plant under in vitro or in vivo conditions. An improved artificial seed production technique is considered a valuable alternate technology of propagation in many commercially important crops and a significant method for mass propagation of elite plant genotypes. The production of plant clones multiplied by tissue culture and distributed as artificial seeds could be a useful alternative to the costly F1 hybrids for different plant crops. The delivery of artificial seeds also facilitates issues such as undertaking several ways for scaling up in vitro cultures and acclimatization to ex vitro conditions. The development of an artificial seed technique also provides a great approach for the improvement of various plant species such as trees and crops.

Keywords: synseed; sodium alginate; encapsulation; micropropagules and micropropagation

1. 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", are firstly described by Murashige [1]. He defined artificial seeds as "an encapsulated single somatic embryo". An artificial seed was later defined by Gray et al. [2] as "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 [3,4]. 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 [5]. 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) [6–8]. Therefore, artificial seeds can eliminate the acclimation steps necessary in micropropagation and give breeders greater flexibility [9]. Various plant materials have since been used for artificial seed production including somatic embryos, shoot tips, auxillary buds, nodal segmants, protocorm-like bodies (PLBs), microshoots, and embryogenic calluses [6,10–14]. 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 [5,13,15–30], as cited in [29].

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 [31]. For example, the success of encapsulation of dormant vegetative buds of an in vivo-cultured mature mulberry tree has been reported by Pattnaik et al. [32]. Furthermore, the production of artificial seeds from encapsulated *Curcuma amada* microshoots has been reported by Banerjee et al. [33], as cited from [31].

2. 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 [2,6]. 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 [34].

Zygotic embryos are formed from the sexual recombination of male and female gametes. Thus, cuttings or other vegetative means are used for propagation and these methods rarely present convenient storage. Artificial seeds could be a good tool to propagate these types of plants and to store their propagules for a reasonable period of time [11]. 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 [8,35]. 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 (Figure 1). Artificial seeds can be also used in the proliferation of male or female sterile plants for hybrid seed production [8]. 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. 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 [36]. Moreover, 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 [7,37]. 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 [6]. Furthermore, artificial seeds are more durable for handling, transportation, and storage. 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 (Figure 1).

Artificial seed production may offer a tool suitable for the extensive scale-up required for multi-clone commercial production [38]. Moreover, the use of this technique economizes upon the space, medium, and time requested by the traditional tissue culture methods [16]. Artificial seed production has great advantages in comparison with traditional tissue culture methods. 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 [39,40].

Artificial seeds can be very useful for grass species as well as many others. 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

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. 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 [1]. The use of tissue culture and micropropagation techniques with several plant species were summarized by [2]. However, more investigations are required to find out the possibility to develop the micropropagation systems to produce artificial seeds.

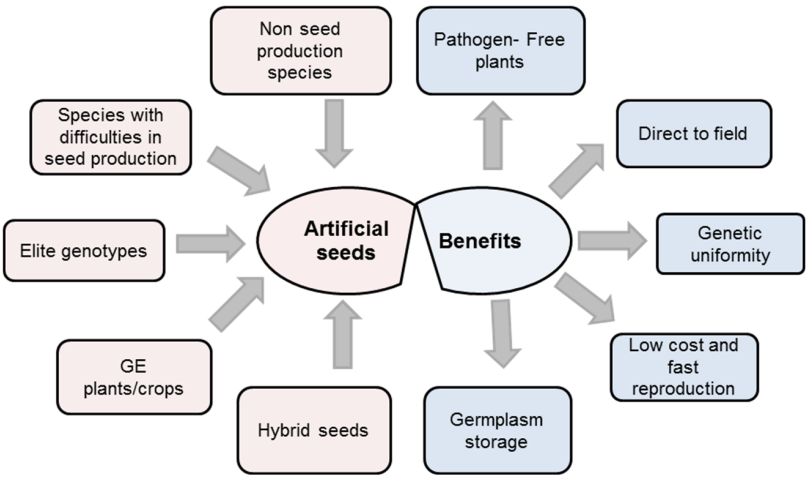

Figure 1. Artificial seed uses and benefits (modified from [41]).

3. 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 [35] (Figure 2).

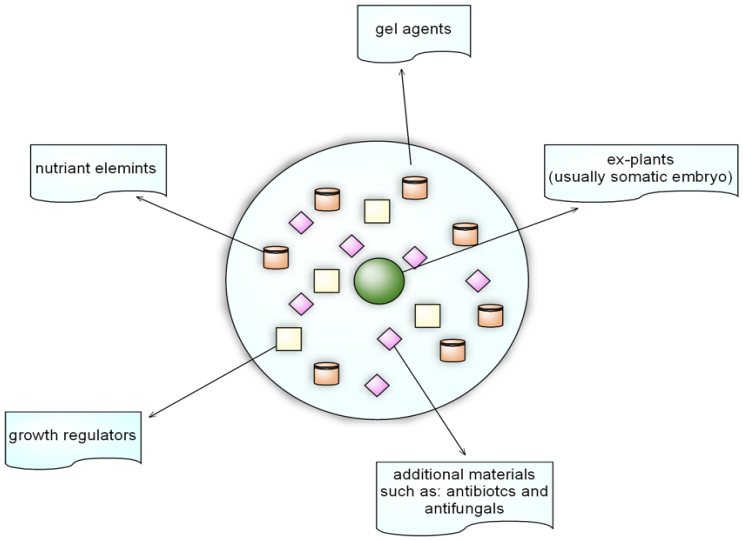

Figure 2. Artificial seed concept.

4. Essential Requirements for the Production of Artificial Seeds

4.1. Explant Material

Explant materials are the basic generative component of the artificial seed. It could be:

4.2. 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 [6]. 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 [42], because it avoids the dedifferentiation callus stage, and consistent genetic structure production [43].

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 including carrot (*Daucus carota*) [44], alfalfa (*Medicago sativa*), Norway spruce (*Picea abies*) [45], sandalwood [45], pistachio (*Pistacia vera*) [46], sandalwood (*Solanium album*) [47], grape (*Vitis vinifers*) and mango (*Mangifera indica* L.) [48], *citrus reticulata* [49], *Hopea parvitflora* [50], *Paulownia elongata* [51], sugarcane [52], *Oryza sativa* (hybrid rice) [53], *Rotula aquatic* (takad) [54], *Daucus carota* (Latif et al., 2007), *Pinus radiata* [55], *Nothofagus alpina* [35], *Catharanthus roseus* (L.) G. Don [56], and many others (Table 1).

However, while Attree et al. [57] indicated that the somatic embryos of white spruce (*Picea glauca*) survived desiccation and grew to plantlets more strongly than its removed zygotic embryos cultured in vitro, Lulsdorf et al. [58] mentioned that the germination level of encapsulated white spruce (*Picea glauca*) and black spruce (*Picea mariana*) had reduced frequency in comparison with the correspondent zygotic embryos. Cartes et al. [35] reported that the encapsulated somatic and zygotic Rauli-Beech (*Nothofagus alpine*) embryos had the same germination habits, which depended on the type of encapsulation applied, and they mentioned that the germination level of zygotic embryos was higher in comparison to somatic embryos. An automatic production and encapsulation artificial seeds protocol was established by [9]. These authors mentioned that the optimal sowing state such as in a nursery bed in the field or in the greenhouse provides a high and homogeneous conversion of artificial seeds. They demonstrated that the conversion level of celery and carrot embryos can be raised from 0% to 53–80% by applying three essential treatments: (1) Culturing the embryos in medium culture with high osmolarity for 7 days: the embryo size was increased from 1–3 mm to 8 mm, and the chlorophyll was also increased during this treatment; (2) the embryos water content was reduced from 95–99% to 80–90%; and (3) post-dehydration culture on SH medium, containing 0.01 mg·L^{-1} GA_3, 0.01 mg·L^{-1} BAP, and 2% sorbitol. The bead quality was also modified by adding 3% sucrose and a mixture of fungicides. It is believed that treating the embryos with high osmolarity at the entail culture stage helps to reduce the water content of the embryos. Moreover, this helps the embryos to acclimate to the new encapsulation conditions.

Ganapathi et al. [59] reported a conversion rate of 66% of *Musa* spp. artificial seeds produced by the encapsulation of their somatic embryos. Artificial seeds of *Paulownia elongata* were produced by the encapsulation of somatic embryos with a conversion rate that was assessed to be 50.3% [51]. A 26% conversion rate was obtained by culture-encapsulated somatic embryos of *Quercus robur* in P_{24} medium supplemented with 0.1 µM IBA, 0.9 µM BA, and 3% sucrose [51]. Kumar et al. [53] reported a 47% conversion rate of *Oryza sativa* cultured in Whatman filter paper. A 100% conversion rate of *Rotula aquatic* artificial seeds produced by the encapsulation of its somatic embryos was achieved when the artificial seeds were cultured in MS medium [54]. Malabadi and Staden [60] and Manjkhola et al. [61] reported conversion rates of 89% and 60.6% of encapsulated somatic embryos of *Pinus patula* and *Arnebia euchroma*, respectively. The encapsulated somatic embryos of *Acca sellowiana* had a high conversion rate that was about 80% after 1.5-years storage [62]. The cultivation of

encapsulated somatic embryos of *Psidium guajava* in MS medium supplemented with 3% sucrose showed a high conversion rate assessed to be 91.6% [13].

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) [63,64], cited in [35]. It was also suggested that encapsulation can affect embryo respiration (Redenbaugh, 1990) and this in turn might influence the germination and viability of somatic embryos [65]. 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 [66,67] cited in [35].

Two types of artificial seeds (encapsulated somatic embryos) are commonly produced: desiccated and hydrated [6,68].

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 [6]. These types of artificial seeds can be only made in plants whose somatic embryos are desiccation-tolerant [31].

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. [69]. 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 [70].

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 [6]. Encapsulation has been expected to be the best method to supply protection and to convert the in vitro micropropagules into 'artificial seeds' or 'synseeds' [71], 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 [72].

- 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 [17] and mulberry [73] into plantlets without specific induction treatment. Ganapathi et al. [17] 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 [74] 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) [75]. Lata et al. [38] 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 [11,76].

- 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 et al. [46] 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 by [77]. 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.

5. 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) [6].

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 [44]. Later applied to alfalfa embryos, Redenbaugh et al. [78] 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 [6,8]. The major principle for alginate encapsulation formation depends on the exchange ions between Na+ in sodium alginate with Ca+ in $CaCl_2 \cdot 2H_2O$, which happens when sodium alginate droplets involving the artificial embryos or any other plant propagule is dropped into the $Ca_2 \cdot 2H_2O$ solution, producing stable explant beads. The solidity and rigidity of the capsule (explant beads) depends upon the two gelling agents' (sodium alginate and $CaCl_2 \cdot 2H_2O$) concentrations and mixing duration. Nutrients should also be added to the artificial endosperm in order to maintain germplasm survival [49], obtain faster explant growth [79], and supply the energy required for germination, which is normally provided by endosperm or gametophyte tissue in true seeds [80]. 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 [8]. However, there are many other materials such as pesticide, antibiotics, and fungicide, which have positive effects in the capsule features.

6. 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 [53,60,81]. While Saiprasad [8] mentioned that usually 3% sodium with complexing solution

containing 75 mM $Ca_2Cl_2 \cdot 2H_2O$ for half an hour mixing duration provides the optimal structure for artificial seed bead formation, Ara et al. [6] indicated that generally 2% sodium alginate gel upon complexation with 100 mM CaCl2·2H2O 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 et al. [7] 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 (Table 1).

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 [82,83]. 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 [84] but also the carbon source [49] and growth regulators to control and sustain growth through germination [85]. 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 [53,60,81].

Artificial endosperm has a great effect on the germination level. In this way, Ara et al. [48] 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 (Table 1). Lakshmana Rao and Singh [86] indicated that the reduction in germination level of encapsulated somatic embryos of hybrid *Solanium-melongenal* 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 [46].

7. Artificial Seeds Storage Ability

Several studies have investigated the artificial seed's storage capability (Table 1). Rai et al. [87] 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 (Table 1). Working with *Rauvolvia serpentine* and applying three different temperature degrees (20 °C, 12 °C, and 4 °C), Ray and Bhattacharya [88] indicated that 4 °C, where storage achieved up to 14 weeks with high regrowth percentage (Table 1), 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.) (Figure 3)—long storage can be achieved using dehydration and/or cryopreservation techniques (Figure 3).

Fabre and Dereuddre [89], 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 [14]. Furthermore, artificial seed conservation facilitates the exchange and distribution of trait plant germplasm, decreasing the requirement for transferring and subculturing out of season [24,62].

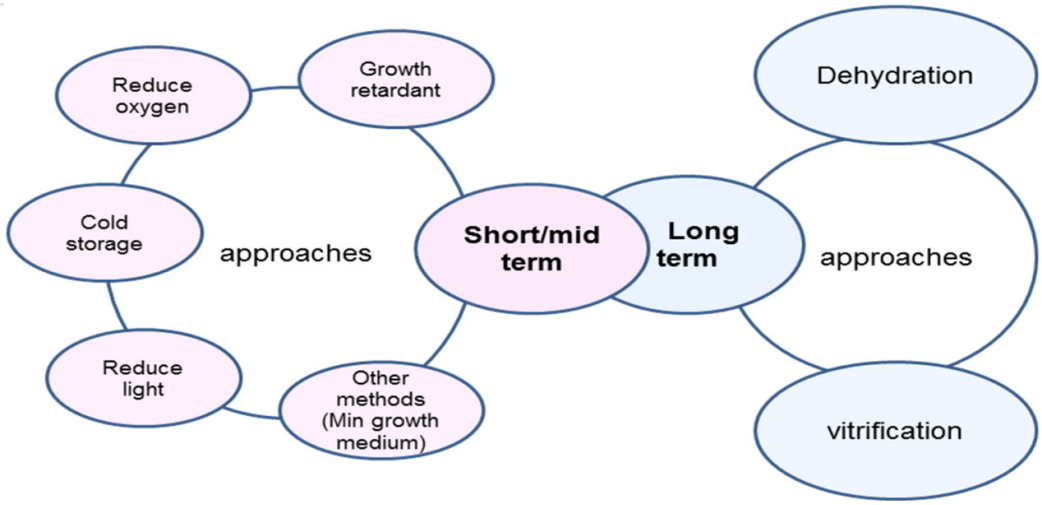

Figure 3. Artificial seed storage approaches (modified from [41]).

Table 1. The recent applications of artificial seeds on different types of plants.

Reference	Species and Explant Used	Pre-Encapsulation Culture	Capsule Structure	Additional Materials	Storage Conditions	Post Storage Treatment or Culture	Storage Duration	Experiment Assessment
[90]	Apical segments of Turkey oak (*Quercus cerris* L.)	Modified GD medium [91] 0.88 BAP + 7.5 g·L^{-1} agar	4% (*w*/*v*) SA immersed in 1.4% (*w*/*v*) $CaCl_2$ for 10 min	GD modified medium + 0.88mM BAP	4 °C	-	6 weeks	85% regeneration and 95% viability
[92]	Shoot tips of azalea (*Rhododendorn simsii* Planch.)	Modified (WPM) medium + (0.3, 0.45, 0.6 M) sucrose (24) h	drops SA immersed in $CaCl_2$ for 15 min	1.62 M glycerol + 0.32 M sucrose (cryoprotectants)	Dehydration to WC (38.6)% + transfer to LN	Detoxification in (1) M sucrose for (2) h	24 h	40% survived after thawing
[24]	Nodal segments of promegranate (*Punica granatum* L.)	-	3% Sa + 100 mM $CaCl_2$ for 30 min	MS medium + 4.44 mM (BA) + 0.54 mM (NAA)	4 °C	(MS) medium + 4.44 mM (BA) + 0.54 mM (NAA)	30 days	The nodals were capable of sprouting.
[93]	Nodal segments of *Rauvolfia tetraphylla* L.	MS medium + 10 mM BA + 0.5 mM NAA + 3% (*w*/*v*) sucrose + 0.8% (*w*/*v*) agar	3% SA + 100 mM for 30 min	MS medium	4 °C	MS medium + 10 mM BA + 0.5 NAA + 3% sucrose + 0.8% agar	4 weeks	Shoot proliferation 86.7%
[94]	Shoot buds were derived from lam (*Progonatherum paniceum*) calli	MS medium supplemented with 2.0 mg·L^{-1} BAP and 0.2 mg·L^{-1} NAA.	3.0% SA + 2% $CaCl_2$ for 20 min	1% activate carbon (*w*/*v*) + 0.3% bavistin (*w*/*v*) + MS medium containing 3% sucrose	-	substrata containing 8 g·L^{-1} agar with half-strength MS medium and 2% sucrose	-	61.58% conversion rate
[95]	Node explants of mugwort (*Artemisia vulgaris*)	Nodal segments were excised from poliferating microshoots	2% SA + 50 mM $CaCl_2$ for 15–25 min	-	5 °C	MS + 3% (*w*/*v*) sucrose	60 weeks	85% of encapsulated nodal segments survived on poliferation mendium
[88]	Shoot tips of *Rauvolfia serpentina*	MS medium + 3% sucrose+ 0.8% agar	3% SA + 100mM $CaCl_2$ for 45 min	Encapsulated shoot tips kept in vessels containing MS + 3% sucrose during storage period	4 °C	MS + 3% scrose	14 weeks	(68.5–100%) regrowth rate during storage period (several tests in the normal culture conditions)
[87]	Somatic embryos of guava (*Psidium guajava* L.)	-	2% SA + 100 mM $CaCl_2$ for 20–30 min	Encapsulated somatic embryos + MS + 9% sucrose or + MS+ 1 mg·L^{-1} ABA	25 ± 2°	MS + 3% sucrose	60 days	28.8% germination level and 37.5% germination level For (sucrose and ABA respectively)
[35]	Somatic embryos from mature seeds of rauli-beech (*Nothofagus alpine*)	-	3% SA + 5.5 g·L^{-1} $CaCl_2$ for 30 min	MS + 0.5 mg·L^{-1} IAA + 0.5 mg·L^{-1} NAA + 2 mg·L^{-1} BAP + 30 sucrose	-	culture MS + 30 g·L^{-1} sucrose + 7 g·L^{-1} agar	-	The germination rate was 45% after 4 weeks
[38]	Axillary buds of *Cannabis sativa* L.	MS medium + 3% (*w*/*v*) sucrose + 0.8% agar + 0.5 mM (TDZ)	5% SA + $CaCl_2$ for 30 min	MS + 0.5 mM (TDZ) + 2.5 mM (IBA). A + fungicide + 5% (PPM)	-	MS medium + 0.5 TDZ + 0.075% PPM	-	100% artificial seed conversion rate

8. Limitation of Artificial Seeds

The main requirement for an efficient artificial seed production protocol is the large-scale production of highly valuable micopropagules 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 oleraceae* var. *botrytis* L.) [11,76,96–98], the micropropagation 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 [31], 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, [99] and the reduction of viability and plant recovery when the artificial seeds are stored at low temperature [100].

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 [101]. 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 [76,102].

9. 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.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. Hail Z. Rihan wrote the entail version of the article. Mohammed E. El-Mahrouk and Fakhriya Kareem updated the article and helped to include most recent achievements in the field of artificial seed production. Michael P. Fuller edited and supervised the work throughout the writing and preparation process.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

SA	sodium alginate
WPM	woody plant medium
BAP	N^6–benzylaminopurine
BA	6-Benzylaminopurine
MS	Murashige and Skoog medium
NAA	naphathaleneacetic acid
GD	Gresshoff and Doy medium
IAA	indolacetic acid
LN	liquid nitrogen

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