EPH - International Journal of Agriculture and Environmental Research

ISSN (Online): 2208-2158
Volume 09 Issue 01 March 2023

DOI:https://doi.org/10.53555/eijaer.v9i1.67

SYNTHETIC SEED TECHNOLOGY, APPLICATION AND FUTURE TRENDS

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Abstract-

In nature, seeds are typically the primary method of plant propagation. Some plants can be vegetatively propagated but conventional methods are time consuming, expensive and cannot produce plants at larger scale. Synthetic seed technology could play a significant role in the production of artificial seeds, or Syn-seeds. Somatic embryos lacked crucial auxiliary tissues like endosperm and protective coverings, which made them difficult to handle and store. Two kinds of somatic embryos are known as desiccated and hydrated seeds based on the techniques developed thus far. The goal of this study is to emphasize the historical and current status of the creation of synthetic seeds. The micropropagation method is still one of the main barriers to the advancement of artificial seed technology. Despite the use of somatic embryos for artificial seed generation in a variety of plant species, there are still some significant problems that must be resolved before progress can be made. The synthetic seed process is a godsend for the vegetative multiplication, conservation, & long-term conservation of rare, endangered, and vulnerable species' superior germplasm. The current scenario of artificial seed to advance agriculture innovation still requires more practical directed appliances.

INTRODUCTION –

In nature, seeds are typically the primary method of plant propagation. Due to seed heterozygosity, small size, lack of endosperms, and necessary requirement for fungal infection for germination, seed propagation has not been successful in several crops (Saiprasad, 2001a). Although certain plants may be reproduced vegetatively, traditional techniques are time-consuming, costly, and incapable of producing plants on a wider scale. Synthetic seed technology could play a significant role in the production of artificial seeds, or Syn-seeds, as a crucial alternative to existing traditional techniques for large-scale multiplication and long-term germplasm preservation of beneficial crop types(Potshangbam, 2016). Some plants can be vegetatively propagated but conventional methods are time consuming, expensive and cannot produce plants at larger scale. Production of artificial seeds/synseeds using synthetic seed technology can play an important role as alternative to other conventional methods for large scale propagation and long-term germplasm storage of useful crop varieties. (Gantait et al., 2015b)The somatic embryogenesis, organogenesis, apical bud, protocorm, & protocorm resembling bodies (PLBs) proliferation systems are used to produce plant micro propagules for synthetic seed generation utilizing in vitro culture techniques. Synthetic seeds of several angiosperm and gymnosperm plants have been created using this encapsulation process.

Type of seed – Generally, there are two kinds of seeds that may be used for plant multiplication and assist preserve the life of vegetation in nature:

1. Organic Seed/Natural seed - As a distinct developmental stage of the spermatophyte life cycle, the seed stage of seed plants has features that are not typical of earlier developmental stages. A matured ovule with an embryo and its coat within is considered to be the fundamental component of a seed. The typical seed contains components that are used in the germination process. The endosperm usually contains these chemicals. Thus, endosperm may include a range of elements that are preserved, including proteins, carbohydrates, and lipids. However, in certain plants, the cotyledons contain the reserve food material.
2. Synthetic Seed - Synthetic seeds are characterized as artificially encapsulated somatic embryos, shoot buds, cell aggregates, or any other tissue that may be utilized for planting as a seed and that has the capacity to germinate under in vitro or ex vitro circumstances and that preserves this capacity even after preservation. In the past, the term "artificial seeds" was exclusively used to describe somatic embryos that were economically useful for delivering plants to the field or greenhouses and producing crops.(Gray & Purohit, 1991)

The goal of the current study is to emphasize the historical and current status of the creation of synthetic seeds, the use of various explant propagules for the manufacture of synthetic seeds, as well as the drawbacks and agricultural uses of the technology.

History –

It is difficult to pinpoint where the concept about an artificial seed first emerged. Certainly, individuals responsible for the initial somatic embryo production may have thought of this use (Reinert, 1959; Steward et al., 1958). F. C. Steward (USA) and J. Reinert almost simultaneously discovered somatic embryogenesis in carrots in 1958 (Germany). Distinguished plant physiologist F. C. Steward works at Cornell New York University. However, the idea of employing somatic embryos as a viable propagation mechanism for seed-sown crops did not start to be put out until the early 1970s. Using somatic embryos, (Drew, 1980) actively developed techniques for commercial crop propagation. He recommended using a fluid drilling device to transfer carrot somatic embryos; however, he was only able to grow three units from carrot embryos in a medium devoid of carbohydrates. Through this approach, he was unable to produce numerous plants. He discovered the extremely sluggish rate of growth of plantlets obtained from culture, which was a critical issue. Dumplings of carrot embryos, roots and Cellus were coated with oligoethylene by (Kitto & Janick, 1985). Some embryos made it through the coating and desiccation steps. It's interesting to note that P. S. Rao's team from BARC, Mumbai, stated that artificial seed made from shoot buds may also be employed for plant multiplication. Since somatic embryogenesis is a relatively new field of study in rice, there is a lot of potential for the widespread dissemination of better, elite hybrid (Brar & Khush, 2021).

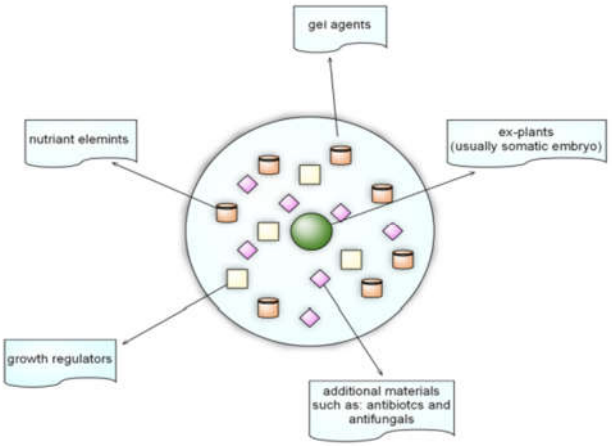
Technology-

The primary barrier to the development of synthetic seeds was that somatic embryos lacked crucial auxiliary tissues like endosperm and protective coverings, which made them difficult to handle and store (Redenbaugh, 1993). Additionally, they are typically thought to lack a silent resting phase and be immune to dehydration. In order to use somatic embryos like a group for clonal plants, the main objective behind synthetic seed development was to create somatic embryos that more nearly mimic seed embryos in storage and handling properties. Germplasm preservation and propagation. Encapsulation technology has advanced as the first significant step in the manufacturing of synthetic seeds in order to achieve this aim. Later, it was believed that the synthetic seeds with encapsulation should also include growth nutrients, microbes that promote plant growth (such mycorrhizae), as well as other biological elements required for the best possible transition from embryo to plant. Another crucial element in the manufacture of synthetic seeds is the selecting of coating material. Two kinds of somatic embryos are known as desiccated and hydrated seeds based on the techniques developed thus far. The somatic embryos used to create the dehydrated synthetic seeds are either left naked or encased in polyethylene glycol before being dried. Desiccation can be accomplished quickly by releasing the pier dishes and letting them on the work bench overnight to dry, or slowly over the course of one or two weeks progressively employing

chambers with decreasing relative humidity. Only species of plants whose somatic embryos are desiccation-tolerant are capable of producing these kinds of synthetic seeds. On the other hand, plants that have somatic embryos that are resistant to desiccation and are susceptible to it generate hydrated synthetic seeds. By placing somatic embryos in hydrogel capsules, synthetic seeds that have been hydrated are created. (Palei et al., 2017)

Artificial seed structure-

The structure of the synthetic seed is similar to that of the natural seed. It is made up of explant material, which resembles the zygotic embryo in a conventional seed, and the capsule, which consists of a gel agent and additional ingredients like nutrient content, growth hormones, anti-pathogens, bio-controllers, and bio-fertilizers and imitates the endosperm in a conventional seed. (Fig.1)



(Fig.1)

Type of synthetic seed-

Different plant propagules are encased with coating materials that serve as synthetic endosperms by providing nutrients to embryos in addition to their protective role. Depending on the many methods used to manufacture them in accordance with the requirements, synthetic seeds may be roughly divided into dehydrated and hydrated seeds.

1. Desiccated artificial seedlings

Somatic embryos are encapsulated with polyoxyethylene and then dried under controlled circumstances. Depending on the situation, desiccation might be done slowly or quickly. While immediate desiccation entails opening a sealed Petri dish holding synseeds and keeping it open overnight for speedy drying, slow desiccation of the encapsulating seeds in a room of decreasing humidity takes one or two weeks (Ara et al., 2000). For plant species with somatic embryos resistant to desiccation, synthetic desiccated seeds can be created.

- Example- By combining measured amount of embryonic suspension with a 5% (w/v) mixture of polyox to create a standard solution of 2.5% polyox, carrot SEs may be encapsulated with polyox. The suspension was applied on telfon sheets by 0.2 ml drop from the pipette, dry to wafer inside a laminar flow hood, and then reapplied. It typically took 5 hours for the teflon plate to dry, depending on how well the water could separate from it. By resolving the wafer in freshly produced embryogenic media and cultivating the rehydrated embryos, the embryo survivability and transformation of seeds were assessed. Abscisic acid (ABA) & mannitol can be added to maturation medium to increase the somatic embryo conversion frequency in celery (Helal, 2011).

2. Hydrated synthetic seed

Hydrogel-encased somatic embryos or appropriate plant tissues make up hydrated artificial seeds. Numerous materials have been investigated, including potassium alginate, agar, gelrite, and sodium pectate, however calcium alginate has been proven to be the most efficient coating material for wet synthetic seeds.(Redenbaugh et al., 1986a)

- Method- The plant components are combined with sodium alginate gel (0.5–10.0% w/v) to create hydrated seeds, which are then pipetted into calcium chloride solution (30–100 mM). As the ion exchange takes occurred and calcium ions replace sodium ions, a round, solid calcium alginate bead is created that contains somatic embryos (Chandrasekhara Reddy et al., 2012).The quantity of sodium ions transferred with calcium ions determines how hard and stiff the capsules are. Therefore, the level of sodium alginate with calcium chloride solution as well as the length of the complexing process may be changed to alter the toughness of calcium alginate gel. When 2% sodium alginate gel was combined with a 100 mM calcium chloride solution, it often resulted in the production of high-quality synthetic seeds for a variety of species of plants (Oceania et al., 2015; Redenbaugh et al., 1986b). Ca-alginate capsules are challenging to handle since they are extremely moist and have a tendency to somewhat clump together. By covering the capsules in Elvax 4260, these issues can be resolved. To prevent bacterial contamination, an antibiotic cocktail including rifampicin, cefotaxime, and tetracycline-HCl could also be included in the matrix (Bekheet, 2006)

Propagules for the formation of synseeds-

Earlier, only somatic embryos were used as explants for the formation of synthetic seeds in a number of plants. However, later reports by various researchers revealed the use of a variety of plant micro propagules, including monopolar axillary shoot tips as well as buds, nodal segments, embryogenic masses, and calla, along with a variety of other explants, including bulb, bulblets, hairy roots, microtubes, and protocorms or protocorm-like bodies (Chandrasekhara Reddy et al., 2012; Gantait & Sinniah, 2012).

1. Somatic embryo –

Somatic embryos are those that are created asexually through somatic cells alone without union of gametes. Plant somatic embryo in vitro development was initially described independently by (Reinert, 1959; Steward et al., 1958). While indirect SEs come from explanted tissues via an intervening callus phase, direct SEs grow directly from explanted cells (Williams & Maheswaran, 1986). However, unlike normal embryos, SEs do not experience desiccation or dormancy and instead begin to germinate as soon as they are completely developed (Zimmerman, 1993). Somatic embryos have been successfully produced in a number of plants thanks to advancements in tissue culture technology, making them more advantageous for the generation of artificial seeds since they are more readily available. If the relative humidity can be kept at 10% like conventional seeds by drying, the SEs can be retained in a viable form for a longer period of time.(Ara et al., 2000).

2. Protocorms and protocorm-like bodies-

When implanted in culture media in vitro, the tiny exalbuminous orchid seeds began to expand after one or two weeks, suggesting effective germination as a result of ingestion of nutrients and water (Nongdam & Chongtham, 2011). The embryos underwent many divisions to become spherules, which are irregularly shaped parenchymatous cell masses (Nongdam & Tikendra, 2014). Protocorms, which are oval, extended, branching, and spindle-shaped creatures that are thought to represent an intermediary structure separating embryos and plants, were formed from the hairy spherical spherules. Although protocorm-like entities are produced in vitro from plant elements other than orchid seeds, they function and morphologically resemble protocorms. Synthetic seeds have been created in a variety of orchid species, including Cymbidium giganteum, Dendrobium wardianum, and Spathoglottis plicata, by encasing protocorms or protocorm-like structures in alginate solution (Corrie & Tandon, 1993; Nagananda et al., 2011; Sharma et al., 1992). When cultivated in vitro on nutritive media or in sterile soil and sand under greenhouse conditions, the encapsulated protocorms of C. giganteum grew into healthy plantlets. In vitro, the frequency of conversion of synthetic seeds was greater than that of seeds that had already germinated in a sand and soil combination. As PLBs have a great potential for direct plantlet formation, (Mohanty et al., 2012) used PLBs to manufacture synseeds in Dendrobium nobile and reported a conversion of synseeds that was notably high at 80%. Coelogyne breviscapa PLBs were encapsulated with a 3% sodium alginate matrix by (Mohanraj et al., 2009) and kept for 60 days before being cultivated into seedlings with Ms medium supplemented including several growth regulators. With longer storage times, synthetic seed germination percentages steadily declined.

3. Calli and Embryogenic Masses-

For the development of clonal plants and for the investigation of genetic change, regenerative and stable embryogenic masses can be employed. However, due to repeated subculturings, keeping them for a longer period of time in bioreactors and culture containers is challenging (Ara et al., 2000). Encapsulating these embryogenic masses in sodium alginate and storing them at 40oC following 6-benzyl amino purine (BAP) treatment might avoid the time-consuming and costly subculturing method (Redenbaugh et al., 1991). The viability and initial proliferative power of synthetic seeds may be kept in storage for around two months. The effectiveness & proliferative nature of embryogenic masses may diminish with an increase in storage time, but further study is needed to determine whether this is the case with synthetic seeds. The growth of progressively randomer lines of cell division, decreased cell specializations, and the disappearance of ordered structures are all related to callus formation (Wagley et al., 1987). Calli's undifferentiated character and minimal differentiation capability restrict their adoption for explant propagules for the manufacture of synseed (Gantait et al., 2015c). For the first time, the use of calli for the formation of synseeds was successfully observed in Allium sativum (Kim & Park, 2002), exhibiting a high rate of conversion and regeneration of synseeds to plants.

4. Apical shoot tips/ shoot buds and nodal segment-

In order to create synthetic seeds, auxiliary shoot buds and/or apical shoot tips that lack root meristems have also been encapsulated. Recoin and Standard documented the encapsulation of shoot tips of apple clone root system M. 26 following an adequate root induction treatment using IBA (24.6 m) over 3-6 days since these structures lack root meristems and need be stimulated to regrow roots before encapsulation. When compare to micropropagation of shoot tip/buds encapsulated synseeds, traditional shoot tip culture in vitro takes more space and culture medium (Gantait et al., 2015c). The need for less space means that moving plant propagules from one location to another is simple.

❖ **Production of synthetic seed-**

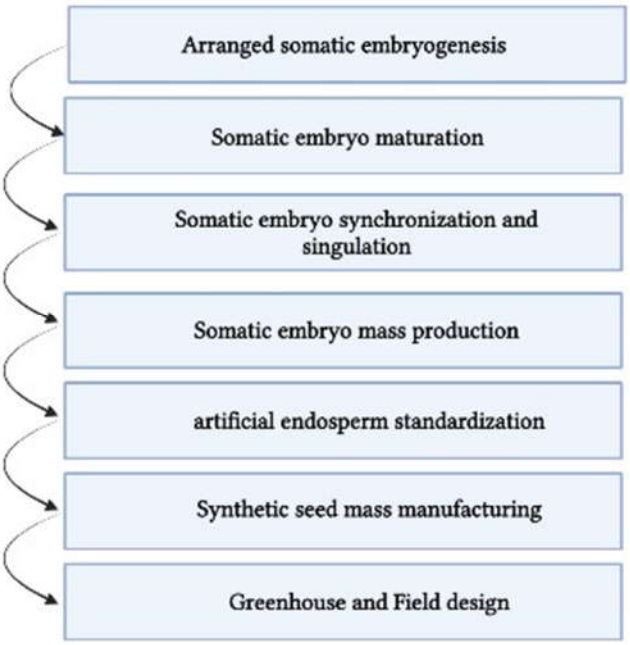
The Production of Commercial Synthetic Seeds Requires the Following Steps

1. Creation of somatic embryos tissue from tissue or cell transformation.
2. Synchronous somatic embryo formation on a large scale.

3. The somatic embryo's maturation.
4. Process for non-toxic encapsulation or coating.
5. Depending on the species, artificial endosperm or a giant gametophyte.
6. The capacity of synthetic seeds to store food.
7. Depending on output, high frequency direct plant conversion from greenhouse or nursery field requirements

❖ **The Events to occur with the Embryo to Plant Conversion Process**

1. Reproduction.
2. Development of strong root system.
3. The growth of shoot merit.
4. The development of genuine leaves.
5. A link from the shoot to the root.
6. The hypocotyls do not have hypertrophy.
7. The hypocotyl's callus development is minimized.
8. A green, phenotypically typical plant
9.



Application of synthetic seed-

Synthetic seed should either lower production costs or raise crop value in order to be beneficial. Whether or not its utilization is appropriate for a particular crop species will depend on the proportionate advantages obtained once development expenses are taken into account.(Saiprasad, 2001)

Numerous plant species may be propagated in vitro or ex vitro (direct sowing), germplasm can be transported and exchanged, and it can be stored for a short, medium, or long time.

1. Propagation –

When transferred to a germination medium, encapsulated explants are distinguished by their capacity for regrowth and conversion following encapsulating & storage at low temperatures (Micheli et al., 2007). Rare and exotic plants, elite genotypes, seedless plants, therapeutic plants, genetically modified plants, and economically significant plants might all be propagated and multiplied using synthetic seeds (Gantait et al., 2015a; Rai et al., 2009). Synseeds may be successfully grown in vitro on semi-solid culture media or planting substrate (such as perlite, vermiculite, soil, soilrite, sand, or gravel) to produce finished plantlets (S. Sharma et al., 2013a). On nutrient-rich media, explants enclosed with calcium alginate beads are generally more efficient at regrowing into full plantlets than it is on nutrient-deficient substrate (Mandal et al., 2000; S. Sharma et al., 2013a). Conversion and complete plant regrowth from encapsulated buds depend critically on the quantity of plant growth regulators in the media (Cheruvathur et al., 2012). The plant species has a considerable impact on the amount of plant growth regulator needed in the nutritional medium. The plant species has a considerable impact on the amount of plant growth regulator needed in a nutritional media. According to (Nishitha et al., 2006), using medium containing 0.49 M IBA and 11.7 M silver nitrate, encapsulate shoots tips of Chonemorpha grandiflora achieved a 95% conversion to plantlets. After becoming used to the soil, plantlets displayed a 90% survival rate. According to (Dhir & Shekhawat, 2013a), Ceropegia bulbosa displayed a maximum percentage response of 100% for the transformation of synthetic seeds into plantlets on medium supplemented with 8.88 M BA. Mondia white encapsulated somatic embryos exhibited a 95.7% survival rate and a 73% germination rate (Baskaran et al., 2014). On semi-solid MS medium containing

10 M mT and 2 M NAA, (Baskaran et al., 2018) produced 91% adventitious shoot regrowth using Urginea altissima encapsulated shoot tips.

2. Short- and Medium-Term Conservation-

The short- and moderate preservation of plant species is one of the options offered by synseeds technology. These procedures are frequently referred to as slow growth methods. The most important criteria to sustain the viability of seeds during transit and conservation are suitable storage environment and a limited storage duration, and these(S. Sharma et al., 2013). The best storage temperature for short- or medium-term varies based on the plant type. The majority of plant species' synseeds have been shown to do best under low temperature storage at 4 C in a laboratory freezer (Ahmed et al., 2015; Alatar et al., 2017; Ray & Bhattacharya, 2008). Numerous scholars have looked at how temperature affects the short- or medium-term storage of synseeds. According to (Faisal et al., 2012), a high conversion rate of 80.6% made 4 ° C the ideal temperature for short-term preservation (storage for up to 4 weeks). After 2 months of storage at 4 ° C. in Ceropegia bulbosa, encapsulated nodal segments converted 50.7% into plantlets, whereas storage for up to 90 days prevented this (Dhir & Shekhawat, 2013b). According to (Faisal et al., 2012), after four weeks of cold storage under low temperatures, overall transformation rate of Withania somniferous synseeds was 86.2%. Additionally, (Khan et al., 2018) discovered that after 4 weeks of storage at 4 C, plantlets from Salix tetrasperma plants encapsulated nodal segments had a 71% conversion and development rate, compared to a 30.33% exchange rate for the same plant's non-encapsulated nodal segments. After one month of storage and after four months of storage, the encapsulated somatic embryos of Curcuma amada cultured at 4 C showed germination rates of 88.10% and 54.16%, respectively (Raju et al., 2016).

3. Transport-

Numerous economically significant plants have been investigated for medicinal, breeding, genetic engineering, and propagation uses. The application of synseed production technology for the interchange and transfer of superior germplasms, axenic plant material, and genetically modified plants between national and international laboratories is possible (Danso & Ford-Lloyd, 2003; Naik & Chand, 2006; Parveen & Shahzad, 2013; Rai et al., 2008; H. Z. Rihan et al., 2017a). According to (Ahmed et al., 2015b), since 4 weeks of preservation at low temperatures, Vitex trifolia's encapsulated nodal segments frequently released their plantlets. This suggests that researchers should investigate the possibility of utilizing this technique for the ex-situ preservation of this forest plant.

❖ **Limitation-**

The development of highly valued micopropagules on a large scale, at a low cost per culture unit, that are appropriate for encapsulation in sodium alginate medium, is the primary prerequisite for an effective artificial seed production process. The micropropagation method is still one of the main barriers to the advancement of artificial seed technology, even if the design of such systems has been accomplished in several species of plants, such as cauliflower.(H. Z. Rihan et al., 2012a, 2017b). Despite the use of somatic embryos for artificial seed generation in a variety of plant species(H. Rihan et al., 2013) has been widely documented, there are still some significant problems that must be resolved to increase the effectiveness of these methods. The benefits of artificial seed technology are offset by drawbacks like storage restrictions brought on by dormancy deficiency, synchronic defect in somatic embryo development, improper maturation, low rates of conversion into plantlets, production restrictions on viable mature somatic embryos, and decreased viability and plant recovery when artificial seeds are stored at low temperatures.(Andrzejewska-Golec & Makowczyńska, 2011).

The idea of employing non-embryogenic propagules as artificial seed generation was examined in several species of plants and found to be a promising option as a propagation approach in species that are resistant to somatic embryogenesis. However, there were some challenges along the road, including it being challenging to get non-embryogenic artificial seeds to take one rooting step (Hung & Trueman, 2011). One of the primary drawbacks of using this technology practically is thought to be the challenges involved in directly planting fake seeds in non-sterile soil or commercial substrates like compost, vermiculite, etc(Jung et al., 2004; H. Z. Rihan et al., 2012b).

❖ **Future trends-**

The synthetic seed process is a godsend for the vegetative multiplication, conservation, & long-term conservation of rare, endangered, and vulnerable species' superior germplasm. With its exceptional aptitude for long-term storage & direct multiplication of seedling to field level, synthetic seed has enormous uses in the area of agriculture in the modern day. This method can restore plant species with valuable elite germplasm with considerable economic and therapeutic values for future generations. Furthermore, plant species that are unable to generate seeds, i.e., seedless species, could be propagated using the synthetic seed technique. Development of synthetic seeds, through direct propagation behaviour from nursery to field, plays a major role in the transfer of prestige plant material from private as well as public laboratories, as well as transport across borders without disease spread via aseptic path. The current scenario of artificial seed to advance agriculture innovation still requires more practical directed appliances. Ongoing efforts have been undertaken over the last few decades, but the relevance on applications to maintain elite germplasm and restore it to its native environment has not yet been successfully channelled. Unfortunately, there is still a collapse of efforts in the notion of the aforementioned rules. Direct planting of synthetic seed in soil or commercial substrates such as compost, vermiculite, and so on is seen as a key barrier for the technique's practical use. Morishige defines synthetic seeds as an encapsulated unique somatic embryo, a clonal product that may be used as real seed for storage, planting, and transport in vitro or ex vitro. Under varied environmental conditions, the encapsulation shell of artificial seed is tested as a protective barrier against drought and infections, and therefore they improve the life span of micro propagules. It is now well known that

the synthetic seed technique is an effective way to transport exceptional germplasm. It also permits polyploidy creation without requiring any genetic recombination, giving it a stronghold in the plant breeding system. In transgenic plants, artificial seed creation using somatic embryos aids in carrying a single gene assigned in a somatic cell and effectively passing it on to progenies with the same aptitude. According to research measures, further research is needed to advance non-embryogenic synthetic seeds to advance culture procedures for their adaption under adverse conditions. For long-term conservation of artificial seed, an in-depth assessment is required, and additional goals must be focused to overcome decreasing survival after storage duration. According to the information presented above, artificial seed is a useful technology for plant propagation. The synthetic seed technique provides a new avenue for long-term preservation of valuable advantaged plant material and aids in the total destruction of endangered, unique, and threatened plant species.(Nandini & Giridhar, 2019)

Conclusion-

Despite significant research investment in artificial seed manufacturing over the past fifteen years, numerous key issues with commercialization persist. The first necessity for the practical deployment of artificial seed technology is large-scale manufacturing of high-quality micro propagules, which is currently a major constraint. Other variables that contribute to poor propagation of synthetic seeds include a lack of oxygen and nutrients microbial invasion, and mechanical injury to somatic embryos. Artificial seeds were successfully generated using encapsulated plant propagules of various plant species. The procedures were optimized, and the appropriate plantlets were obtained. This approach offers several advantages, including a low-cost delivery system, reduced plantlet costs, a straightforward technique with strong potential for scale production, a promising strategy for direct usage of artificial seedling in vivo, and a large storage capacity. The advancements of this procedure are dependent mostly on species of plants in the initial phase. Despite the benefits of artificial seeds, further study is needed to promote root production in non-embryogenic artificial seeds. More research is needed to increase artificial seed culture capability in commercial substrate and under non-sterile circumstances. The synthetic seed technique has enormous potential for micropropagation and germplasm protection; however, further study is required to improve the technique so that it can be utilized commercially.

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