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Review on Production and Application of Synthetic Seeds

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ABSTRACT

Synthetic seeds are artificially encapsulated somatic embryos, shoot tips, axillary buds or any other meristematic tissue, used for sowing. Artificial seeds are advantages hence, better and clonal plants could be propagated similar to seeds; preservation of rare plant species for extending biodiversity, more consistent and synchronized harvesting of important agricultural crops, easy of handling, potential long-term storage and low cost of production and subsequent propagation are among. Desiccated and hydrated seeds are common synthetic seeds and main purpose of synthetic seed is to overcome problem of developing inbreed lines for self incompatible plants like fruits and ornamental plants. There are number of possible artificial seed production systems, depending upon the type of artificial seed produced, need of artificial seeds, the economic feasibility and species. Costly for production, inefficient germination and lack of dormancy are common limitations of artificial seed.

Key words: Encapsulation, Somatic embryo, Synthetic seed

1. INTRODUCTION

Seed (or zygotic seed) is the vehicle that connects one generation to another in much of the plant kingdom. By means of seed, plants are able to transmit their genetic constitution in generations and therefore seeds are the most appropriate means of propagation, storage and dispersal (Bewley J.D. *et al* 1985). An artificial seed is often described as a novel analogue to true seed consisting of a somatic embryo surrounded by an artificial coat which is at most equivalent to an immature zygotic embryo, possibly at post-heart stage or early cotyledonary stage (Bekheet S.A., 2006). In general, synthetic seeds are defined as artificially encapsulated somatic embryos, shoot tips, axillary buds or any other meristematic tissue, used for sowing as a seeds and possess the

ability to convert into whole plant under *in vitro* and *in vivo* conditions and keep its potential also after storage (Capuano *et al*., 1998).

The idea of synthetic seeds or artificial seeds was first conceived by Murashige in 1977. Initially, the development of synthetic seeds had been restricted to encapsulation of somatic embryos in a protective jelly. It had been considered that the induction of somatic embryogenesis (SE) and/or pollen embryogenesis which genetically differs from zygotic embryogenesis is the prerequisite for the preparation of synthetic seeds (Nor Asmah, H., 2011). Artificial seeds have great potential for large scale production of plants at low cost as an alternative to true seeds (Roy B. and Mandal A.B., 2008). There are various advantages of artificial seeds such as; better and clonal plants could be propagated similar to seeds; preservation of rare plant species extending biodiversity could be realized; and more consistent and synchronized harvesting of important agricultural crops would become a reality, among many other possibilities (Khor E. and Loh C.S., 2005). In addition; easy of handling, potential long-term storage and low cost of production and subsequent propagation are other benefits (Bekheet S.A., 2006).

The artificial seed production technique was first used in clonal propagation to cultivate somatic embryos placed into an artificial endosperm and constrained by an artificial seed coat. Today artificial seeds represent capsules with a gel envelope, which contain not only somatic embryos and apical buds or stem and root but also axillary segments (Vdovitchenko Y.M. and Kuzovkina I.N., 2011). Explants such as shoot tips, axillary buds and somatic embryos are encapsulated in cryoprotectant material like hydrogel, alginate gel, ethylene glycol, dimethylsulfoxide (DMSO) and others that can be developed into a plant.

The coating protects the explants from mechanical damage during handling and allows germination and conversion to occur without inducing undesirable variations. They behave like true seeds and sprout into seedlings under suitable conditions (Asmah N.H., 2011). However, as described in literature, the major stumbling block in establishing artificial seed production as a viable technology is a lack of understanding of the SE process and an inability to consistently produce high-quality propagules that can germinate in a soil environment with an acceptably high success rate **(**Pond S. and Cameron S., 2003). Objective of this paper is reviewing production and application of artificial / synthetic seeds.

2. PRODUCTION OF ARTIFICIAL SEEDS

Many studies have been conducted on synthetic seed production in horticultural crops, but the efforts in field grown crops are limited. So, there is a greater scope for synthetic seeds in commercial crops and ornamental plants (Biradar S., 2003).

2.1. The need for artificial or synthetic seeds

Seed is basically zygotic embryo with enhanced nutritive tissues and covered by several protective layers. Seeds are desiccation tolerant, durable and quiescent due to protective coat. Such properties of seeds are also used for germplasm preservation in seed repositories. Zygotic embryo seeds are the result of sexual reproduction that means the progeny of two parents. This has led to the development of often complex breeding programs from which inbred parental lines are developed. Such inbred lines are used to produce uniform hybrid progeny when crossed (Dhabhai R. and Prakash A., 2012).

Primary problem associated with such seeds is, on one hand for many crops, such as fruits, nuts, and certain ornamental plants; it is not possible to produce a true-breeding seed from two parents due to genetic barriers to selfing. Other important criteria relate to the type of artificial seeds that can be produced. Seeds of some tropical crops are recalcitrant (unorthodox) in that they have short viability and must be stored at relatively high moisture content to maintain viability (Bewley and Black, 1985). Therefore, for such crops, propagation is accomplished either vegetative by cuttings or the use of relatively low equality open pollinated seed is tolerated (Keith R., 1990).

After the discovery of somatic embryogenesis in 1950 it was possible to have an alternative of conventional zygotic seeds. Somatic embryo arises from the somatic cells of a single parent. They differ from zygotic embryos since somatic embryos are produced through *in vitro* culture, without nutritive and protective seed coats and do not typically become quiescent. Somatic embryos are structurally equivalent to zygotic embryos (Dhabhai R. and Prakash A., 2012).

2.2. Types of artificial seeds

According to the available literature, two types of synthetic seeds were developed, that is, **desiccated** and **hydrated** synthetic seeds (Bhojwani and Razdan, 2006). The desiccated synthetic seeds were first introduced from somatic embryos either naked or encapsulated in polyox followed by their desiccation (Kitto and Janick, 1982, 1985). Desiccation was achieved

either slowly over a period of one or two weeks sequentially using chambers of decreasing relative humidity or rapidly by leaving the Petri dishes overnight on the bench of laminar airflow chamber (Ara *et al*., 2000).

The hydrated synthetic seed technology was first produced by encapsulating hydrated somatic embryos of *M. sativa* (Redenbaugh et al., 1984). These hydrated synthetic seeds are used to produce plant species that their somatic embryos are recalcitrant and sensitive to desiccation. Hydrated artificial seeds are normally prepared by encapsulating the somatic embryos or other propagules in a hydrogel capsules. Several methods have been examined to produce hydrated artificial seeds of which calcium alginate encapsulation has been mostly used (Redenbaugh *et al*., 1993). Synthetic seeds in plant propagation were successfully studied in number of the plant species. Plant propagation using artificial or synthetic seeds derived from somatic embryos or other vegetative propagules opens up new vistas in agriculture and forestry. Here, according to the convenience and importance of the plant species, artificial seed technology was developed and categorized into different groups.

Cereals: The application of synthetic seed technology to the cereals started from the year 1989. Most of investigations were carried out to increase their yield and vigor. Artificial seeds are playing a major role in increasing the genetically transformed plant material and haploid plant production. George and Eapen (1995) reported the encapsulation of somatic embryos in *Eleusine coracana*. Suprasanna *et al*. (1996) showed that the encapsulation of somatic embryos and conversion into plantlets of *Oryza sativa*. Suprasanna et al. (2002) studied the viability of encapsulated embryos derived from five year old long term culture of *Oryza sativa* cv. *basmati* 370.

Arunkumar *et al*. (2005) reported the addition of protectants, bavistin and streptomycin as constituents of synthetic endosperm and found that there was no negative effect on germination and conversion. They also studied the conversion of synthetic seeds into seedlings in hybrid rice and reported that the application of self-breaking gel beads technology increased the germination (52%) and conversion (47%) of synthetic seeds. Roy and Mandal (2008) reported the development of synthetic seeds involving androgenic and pro-embryos in elite *Oryza sativa*. Model systems for synchronous somatic embryo production combined with encapsulation to form synthetic seeds were studied in *Zea mays* var. *saccharata* (Thobunluepop *et al.*, 2009).

Vegetables: Production of artificial seeds for different vegetables, were started at different time from different part of the plant. The production of synthetic seeds was by the encapsulation of multiple carrot somatic embryos (Kitto and Janick, 1982). 100% germination of encapsulated axillary buds by adding 0.5 mg/l NAA and 1.0% activated charcoal and advanced synthetic seed production systems by using somatic embryos in *Ipomoea batatas* were reported (Onishi et al., 1992, 1994).

Encapsulation of different explants (somatic embryos, plantlets, cell aggregates from hairy roots), conservation of root regeneration potential of cell aggregates in coated capsules even after stored at 25°C up to 60 days and plant regeneration from them were observed respectively in *Armoracia rusticana* (Nakashimada *et al*., 1995). Encapsulation of somatic embryos, nodal segments, shoot tips and cell suspension cultures and estimation of yield and canopy of field cultivated plants derived from synthetic seeds of *Solanum tuberosum* (Nyende *et al.,* 2005) were investigated.

Phonkajornyod et al. (2004) reported the dry synthetic seed production and desiccation tolerance induction in somatic embryos of *Capsicum annuum*. Encapsulation of nodal segments and shoot tips of *Manihot esculenta* (Cassava) germplasm was reported (Cid et al., 2009). Ummi *et al*. (2011) investigated the effect of different storage intervals on encapsulated embryo and germination of *Parkia speciosa*. The encapsulated *P. speciosa* zygotic embryo (without storage) showed that germination occurred after seven days of culture on the germination medium. The synthetic seed stored at 4°C remained viable and germination is initiated on day 14 after culture.

Fruit crops: In most of the commercial fruit crops, the seed propagation has not been successful because of heterogeneity of seeds; minute seed size and presence of reduced endosperm, low germination rate and in some crops have desiccation sensitive and recalcitrant seeds which cannot be stored for longer time (Rai *et al.,* 2009). Recently many of the crops available are seedless varieties. Propagation of Musa paradisica (Hassanein et al., 2005.) and Musa paradisica cv. grand naine (Sandoval-Yugar *et al*., 2009) was carried out through encapsulated shoot tips. In banana cv. rasthali (Musa spp. AAB group), plantlet regeneration was from alginate encapsulated somatic embryos (Ganapathi *et al*., 2001).

M 26 apple rootstock synthetic seeds prepared by using apical and axillary micro-propagated buds(Brischia *et al.,* 2002). Attempts for saving labor by using mechanical tools in the production of adventitious shoot tips suitable for encapsulation were tried by Sicurani et al.

(2001). Micheli *et al.* (2002) stated that the presence of second layer of alginate (double encapsulation) and of a thin external coating layer on the alginate (encapsulation coating) did not show any detrimental effects on viability, sprouting and re-growth of the encapsulated micro-cuttings in M 26 apple rootstock. Effect of encapsulation on *Citrus reticulata* somatic embryo and their plantlet conversion, prospective of the encapsulation technology in the nursery activity of *Citrus* were studied by different authors (Antonietta *et al*., 2007). As compared to non-encapsulated or encapsulated with a growth regulator free artificial endosperm, somatic embryos encapsulated with an artificial endosperm containing GA3 has greater ability to plantlet conversion (Antonietta *et al*., 1999).

Ornamental plants and orchids: In ornamental plants and orchids, the synthetic seeds have very much commercial importance, because of their minute seed size and presence of reduced endosperm in seeds (Lambardi *et al*., 2006). Ruffoni *et al.* (1994) produced synthetic seeds of somatic embryos in two ornamental species (*Eustoma grandiflorum* and *Genista monosperma*). Piccioni and Standardi (1995) produced synthetic seeds of shoot tips in *Betula pendula and* bulbs in *Lilium longiflorum* (birch).

In orchids the most widely used explants for the preparations of synthetic seeds are the seeds, protocorms and protocorms like bodies (Murthy *et al*., 2006). In the recent years, the researchers are mostly concentrating on the enhancement of protocorm like bodies (PLBs) to make synthetic seed system commercial for the propagation of orchids. Khor *et al.* (1998), developed two-coat system for encapsulation of *Spathoglottis plicata* seeds and protocorms. The seeds and protocorms could withstand the encapsulation treatment with high viability 64 and 40%, respectively.

Forage legumes: among the forage legumes somatic embryogenesis and the development of synthetic seed, technology was extensively studied in *Medicago sativa* (alfalfa). Encapsulation in hydrogel remains to be the most studied method of artificial seed production in alfalfa (Walker, 1990). Induction of desiccation tolerance in *M. sativa* somatic embryos by ABA treatment and production of synthetic seeds were studied by Senaratna *et al*. (1989, 1990). They found that somatic embryos treated with ABA showed about 60% survival and conversion into plantlets when placed on moist filter paper or sown directly onto sterile soil. However, efficient coating and encapsulation methods for desiccated embryos of *M. sativa* are yet to be developed (Redenbaugh *et al*., 1991).

Spices and plantation crops: germination capacity and survival rate of artificial *Coriandrum sativum* seeds were 82% and 83% respectively (Chen *et al.* 1991). Artificial seeds of *Coriandrum sativum were* produced by using somatic embryos derived from hypocotyls explants (Jayabalan, 2000). Production of disease free encapsulated shoot buds of *Zingiber officinale* and their conversion into plantlets were reported by Sharma *et al.* (1994).

Cold storage of shoot cultures and alginate encapsulation of shoot tips of *Camellia japonica, Citrus reticulata* and propagation of tea (*C. sinensis*) by shoot proliferation of alginate-encapsulated nodal explants stored at 4°C were reported (Mondal et al. 2002). Induction of somatic embryo-genesis, production of synthetic seeds and 70% of germination in *Elaeis guineensis* was studied by Mariani *et al*. (2008).

Medicinal plants: naturally most of the important medicinal plants are rare, endangered and endemic category. It is due to the low fruit and seed formation, poor germination capacity of seeds and due to the many other environmental conditions such as habitat modification, urbanization, climatic change and pollution etc. So, it is important to propagate and conserve these plant species. The production of synthetic seeds by encapsulating somatic embryos and vegetative propagules is rapidly becoming an applied technique with potential for mass propagation of medicinal plant species (Reddy *et al*., 2012).

2.3. Procedure for the production of artificial seeds

There could be a number of possible artificial seed production systems, depending upon the type of artificial seed produced, need of artificial seeds, the economic feasibility and it will vary greatly among species (Pond S. and Cameron S., 2003).

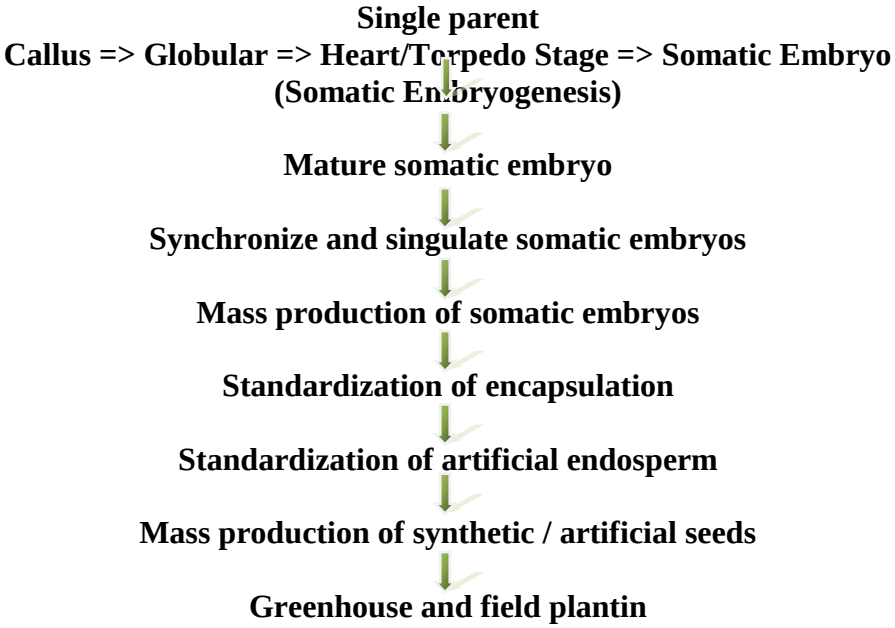

Figure 1. General procedure for production of artificial seed

The development of the ideal viable, quiescent, low-cost artificial seed has been described as a 10- step process (Redenbaugh K. *et al.*, 1987). First of the steps is the selection of the crop based on technological and commercial potential followed by the establishment of a somatic embryo system (species specific). Next is the optimization of the clonal production system, optimizing protocols to synchronize and maximize the development of normal mature embryos capable of conversion to normal plants. Automation of embryo production is followed by this. After that, post-treatment of mature embryos to induce quiescence, development of an encapsulation and coating system, optimization and automation of the encapsulation system and conversion requirements for greenhouse and field growth (watering, fertilizer, transplantation, etc.) are followed (Dhabhai R. and Prakash A., 2012).

Identification and control of any pest and disease problems that may be unique to artificial seeds and determination of the economic feasibility of using the artificial seed delivery system for a specific crop compared with other propagation methods (cost–benefit analysis of encapsulation versus other options) are last steps (Redenbaugh K. *et al.*, 1987). Some steps generally apply to more than one species whereas other stapes may be species-specific.

2.4. Encapsulation of somatic embryos

The somatic embryos isolated are submerged in a solution of sodium alginate, according to the type of encapsulation applied, and subsequently suctioned through a micropipette to provide a protective capsule. In order to seal the capsules, they are then submerged in a complexing solution of $CaCl_2$ for a determined period of time followed by washing in sterile water for 40 min. This process is carried out under aseptic conditions in a laminar flow chamber, laminar with prior sterilization of the material and culture medium.

Finally, the artificial seeds are cultivated in a germination medium in Petri dishes with macro and micronutrient from the MS medium supplemented with 30 g/l of sucrose and 7 g/l of agar-agar. They are then left in the culture chamber at a temperature of 25ºC in complete darkness (Pond S. and Cameron S., 2003).

2.5. Limitation of Artificial or Synthetic Seeds

Several intensive researches in the field of synthetic seed technology were applied in propagating and conserving a number of plant species, but practical implementation of the technology limited due to the following main reasons:

- Production and storage of synthetic seed is costly (production technique itself becomes costlier, production of viable micropopagules useful in synthetic seed production is less.)
- Inefficient germination and conversion in to normal plants due to embryos maturation time variability.
- Seed dormancy problem due to lack of dormancy, stress tolerance in somatic embryos and limited storage of synthetic seeds (Ara *et al*., 2000)

2.6. The Genetic Stability of Synthetic Seeds

Synthetic seeds have been widely used for micro-propagation of many plant species. The molecular studies to determine genetic stability of synthetic seeds derived plantlets were started from the last decade, but no modifications were revealed at the biochemical and/or molecular levels.

The potential advantage of synthetic seeds for genetically identical to natural plants was supported by many reports (Nyende *et al*., 2003). The genetic stability of plantlets derived from encapsulated *Ananas comosus* micro shoots was proved by random amplified polymorphic DNA

(RAPD) and ISSR techniques (Gangopadhyay *et al.,* 2005). Bekheet (2006) reported that in *Allium sativum* both plantlets derived from encapsulated bulblets as well as normally *in vitro* were genetically similar to those that the *in vivo*. RAPD analysis showed the genetic stability of *in vitro* plantlet derived from encapsulated shoot tips of *Dioscorea bulbifera* (Narula et al., 2007). Srivastava et al (2009) reported that *Cineraria maritana*, analysis of the RAPD profiles revealed an average similarity coefficient of 0.944; they confirmed the molecular stability of plants derived from encapsulated micro-shoots followed by six months of storage.

The genetic stability between mother plants and somatic embryo derived synthetic seeds showed resemblance in *Cucumis sativus* and proved by using RAPD markers (Tabassum *et al.,* 2010). In *Picrorhiza kurrooa,* the genetic stability of plants derived from encapsulated micro-shoots following three months of storage was proved by using cluster analysis of RAPD profile (Mishra *et al.,* 2011). Lata *et al*. (2011) reported genetic stability of synthetic seed derived plants of *Cannabis sativa* studied by using ISSR- DAN fingerprinting and gas chromatography (GC) analysis of six major cannabinoids and showed homogeneity in the re-grown clones and the mother plant.

2.7. Applications of Artificial Seeds

Artificial seeds have vast application in different fields of plant biotechnology for cultivation of various plant species. They offer the opportunity to store genetically heterozygous plants or plants with a single outstanding combination of genes that could not be maintained by conventional methods of seed production due to genetic recombination exists in every generation for seed multiplication (Gray D.J., 1997). Many species are sterile and produce no seeds. Somatic embryogenesis is an alternative with respect to the cuttings to propagate these plants. Other species, including some tropical produce recalcitrant seeds that cannot be dried. Consequently, long-term storage in gene banks in these species is not possible. The artificial seeds can be an alternative as more is learned about the mechanism by which this type of seed has no tolerance to desiccation (Leprince O., *et al*., 1993).

In autogamous species, where the production of hybrid seed is difficult and expensive, the artificial seed technology offers many advantages and opportunities. One of the limitations of the method of micro propagation is that they should be in the same physical site of tissue culture laboratories and greenhouses, as production of propagules must be synchronized in periods of

peak demand in the market. Artificial seed production in these species would not link the laboratory facilities of the greenho

ses (Gray D.J *et al*., 1991).

The market for ornamental plants is growing every year. The high cost of production of these species is given by the diligence of the micro propagation and manpower needed in the later stages of propagation and production. The use of somatic embryogenesis system in these species would significantly reduce labor costs (Chee R.P *et al*., 1992).

Coniferous forest species can be propagated cheaply through seeds. The conventional breeding programs in these species are very time consuming because the life cycle of conifers is very long. Coniferous forests are very heterogeneous and that the seed of outstanding individuals will not necessarily give rise to improved offspring. Artificial seed has the ability to clone those over hanging trees at reasonable cost and in minimum time (Desai B.B.,. 1997).

In the commercial sector, it is very difficult to produce low-cost hybrid seed species such as cotton (Gossypium hirsitum L.) and soybean (Glycine max Merril.) because they have cleistogamous flowers and abscission problems as the seed that is currently used comes from self-pollinating species. However, hybrid seed is produced in small quantities in a very laborious by hand pollination. This small volume of hybrid seed could be massively increased through artificial seed technology. Thus, the hybrid force would be used commercially to originate a significant reduction in costs (Tian I. *et al*., 2000).

In certain vegetable species, used hybrid seeds are expensive and therefore the plant value is very high. For example, tomatoes and seedless watermelon hybrid seeds are used in very cost. The reason for this high cost is that pollination is done by hand, requiring intensive labor. In other species, vegetative reproduction is used it also consumes much time, space and labor. The use of artificial seed technology can significantly reduce costs by reducing the labor required, time and space in case of these plants (Chee R.P. and Cantliffe D.J., 1992).

Sowing seed of synthetic varieties is a common practice in forage species such as alfalfa (*Medicago sativa* L.) and orchar grass (*Dactylis glomerata* L.). Such varieties from selection and crossing of lines are phenotypically uniform but different genotypes. These lines to cross freely year after year to produce seeds, heterozygous and heterogeneous populations originate. The use of artificial seed allows multiplication of outstanding genotypes and genetically uniform, since

this method does not require that annually cross-pollination is carried out to produce plants (McKersie, B.D. and Brown D.C.W., 1996).

The vast majority of fruit species are propagated by vegetative means because of the presence of self-incompatibility and breeding cycles very long. The use of synthetic seed facilitates its spread. However, the most useful artificial seed would be in the conservation of germplasm of these species (Towill L.E., 1988). Currently seed banks are maintained as live plants in the field. This method of conservation is very expensive and dangerous, as it is exposed to natural disasters. The use of artificial seeds would retain these clones in a small space, under controlled conditions (cryopreservation) and without the danger of natural disasters. In addition, this system of germplasm conservation would be particularly useful in tropical species where conservation means are inadequate or nonexistent. The vine (*Vitis* spp.) is a practical example of this system of conservation.

In cross-pollinated species like maize, where production of hybrid seed is a widespread practice. The creation of hybrids through a conventional breeding program consumes much time and resources in obtaining and maintaining appropriate parental lines. One possibility is the use of artificial seed to propagate outstanding genotypes without the need to generate parental lines costly in time and money. This could facilitate the commercialization of new hybrids and encourage the emergence of new seed companies, as it would be possible to produce new hybrids without the need for large amounts of parental lines (Desai B.B. *et al.*, 1997).

In autogamous species such as wheat, barley and oats where hybrid seed production at commercial level is not possible by high production costs, artificial seeds would spread the hybrid seed. In this case, produce small quantities of hybrid seed by hand and then with the technology of artificial seed multiplication would be carried out mass (Kumar, U., 1998).

There are a growing number of species that are in the process of extinction. Indiscriminate felling of forests, increasing desertification, disappearing forests, etc. increases the changes of extinction of species. Many of these native species cannot be propagated vegetatively, or produce very low quantities of seed. For this reason, the artificial seed is an alternative for these species. Crops from genetically modified plants have boomed in recent years. There is little information about what happens to these GMOs in the process of sexual reproduction. It is possible that during sexual multiplication, the introduced genes from other species are meiotic ally unstable and lost. With the use of artificial seed technology would avoid such risks.

Similarly, this technology could be used in the propagation of somatic hybrids and cytoplasm (obtained through protoplast fusion) and in sterile and unstable genotypes (Kumar, U., 1998).

3. SUMMARY AND CONCLUSION

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

Synthetic seeds technique is a rapid tool of plant regeneration because of its wide use in conservation and delivery of tissue cultured plants. Protocols of encapsulation were already optimized for various plant species, but the commercial scale production of synthetic seeds was restricted to few species only due to several major problems, such as: asynchronous development of somatic embryos, improper maturation of somatic embryos, poor conversion rate of somatic embryos, lack of dormancy, and limited production of viable mature somatic embryos.

Development of protocols for direct recovery of plants from synthetic seeds under non sterile conditions may have a greater impact. Although large number of plants can be produced in tissue cultures through embryogenesis / multiple shoot cultures, their delivery is cumbersome. Embryos or shoots have to be separated singly and transferred for rooting to achieve root shoot balance, and the plants have to be hardened in the green house before field planting.

Direct sowing of synthetic seeds in the soil does not need acclimatization often required for the tissue cultured plants. It thus provides an ideal delivery system enabling easy flexibility in handling and transport as compared to large parcels of seedlings or plants. For large scale commercialization in synthetic seeds technology, enhanced production of propagules is necessary. Current tissue culture methods do not generate adequate propagules and are not sufficient to meet the demands of commercial exploitation of synthetic seeds technology. Standardization of methods for synchronization of developing propagules followed by automation of the whole process of sorting, harvesting, encapsulation and germination of the coated propagules can enhance the pace in the production of synthetic seeds.

However further detailed research is needed mainly for improvement in conversion frequency of synthetic seeds and subsequent plantlet growth in soil. Although results of intensive researches in the field of synthetic seed technology seem promising for propagating a number of plant species, practical implementation of the technology is constrained due to the following main reasons:

- Limited production of viable micro-propagules useful in synthetic seed production.
- Improper maturation of the somatic embryos that makes them inefficient for germination and conversion in to normal plants.
- Lack of dormancy and stress tolerance in somatic embryos that limit the storage of synthetic seeds.
- Poor conversion of even apparently normally matured somatic embryos and other micro-propagules into plantlets that limit the value of the synthetic seeds and ultimately the technology itself.

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