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Synthetic Seed Technology in Horticultural Crops for Conservation and Utilisation of Germplasm

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Abstract **– In different plant species, artificial seeds are produced successfully by encapsulating the plant propagules. This technique has been considered to be advantageous since it has cost effective delivery system, minimization of the cost of plantlets, simple methodology with high potential for mass multiplication and production, a promising technique for the direct use of artificial seedlings in vivo and a high storage capacity. This review paper emphasizes the feasibility of using somatic embryos for conservation of germplasm. It also encourages for optimizing the encapsulation matrix for large scale preparation of synthetic seeds. In plant biotechnology, the methods for encapsulation and somatic embryogenesis technologies creates an innovative and potential tool for an efficient and cost effective large scale propagation, breeding, in vitro conservation, non embryonic synthetic seed production and exchange and distribution of germplasm. It has been reviewed and verified in this paper that the production and development of synthetic seed technology is an authentic method of propagation in several commercially important agronomic and horticultural crops and has also been suggested as a powerful tool for mass propagation of elite plant species with high commercial value. These synthetic seeds would also be a channel for new plant lines produced through biotechnological tools to be delivered directly to the greenhouse or field and is also considered to be a high volume, low cost production technology, easy to transport, easy of handling while in storage, has a potential for long term storage without losing viability, rapid multiplication and thereby maintaining genetic uniformity of plants.**

Keywords **– Synthetic Seed, Encapsulation Technology, Horticultural Crops, Mass Multiplication, Conservation.**

I. INTRODUCTION

Synthetic seed is one of the most promising tools of plant biotechnology, which could be tailor-made for horti- and agricultural improvement at present as well as upcoming days. As all the propagules used for synthetic seed preparation are produced through *in vitro* clonal propagation, which means they did not encounter two fundamental events of sexual reproduction, the meiotic recombination (during crossing over) and gametic fusion of two different parental genome (cross-pollination), both of these events can create new types of heterozygosity in zygotic seeds. Therefore synthetic seed-derived offspring are always true to type to their source plant. Synthetic seeds have multiple advantages for propagation including ease of handling, higher scale-up potential, low cost production, disease eradication, and potential medium- and long-term conservation. For some plants such as ornamental plants, propagation through somatic embryogenesis and artificial seeds is the only source of conservation.

II. HISTORY AND EVOLUTION OF SYNTHETIC SEEDS

Use of *in vitro* somatic embryogenesis to produce "synthetic seeds" offers the potential of suitably efficient vegetative propagation. Somatic embryos form continuously in cell cultures and production of several thousand embryos per gram of culture material has been achieved. Furthermore, somatic embryos are morphologically and, in most respects, developmentally analogous to zygotic embryos found in seeds. For example, somatic embryos possess typical embryonic organs and can germinate bipolarly as do their zygotic counterparts.

However, somatic embryogenesis has yet to gain utility as a means of plant propagation despite the fact that it has been recognized for over 35 years and has been documented for over 200 crop species. Only in recent years have attempts been made to confer seed-like storage and handling qualities on somatic embryos through development of synthetic seed technology. Broadly defined, synthetic seed consists of somatic embryos processed to be of use in the production of a particular crop. For some crops, this could constitute hand manipulated, naked embryos reared in callus cultures. However, quiescent somatic embryos, encapsulated in synthetic seed coats and produced in mass by an automated process will be required for others. The purpose of this review paper is to discuss the applications and current developmental status of synthetic seed technology for horticultural crop production. The concept of production and utilization of synthetic seeds (somatic embryo as substitutes for true seeds) was first suggested by Murashige in 1977 (Bajaj, 1995, Cyr, 2000). The origin of the idea of an artificial seed is difficult to trace, certainly those who first produced somatic embryos may have considered such an application, simultaneously discovery of somatic embryogenesis in carrot was reported by Steward *et al* (1958) and Reinert (1959). Steward, a renowned plant physiologist, at Cornell University in New York, was impressed by the dramatic effects of coconut milk in carrot culture media, growth factors and liquid endosperms. Among the active materials he extracted from the coconut milk that are now commonly included in purified form in many tissue culture studies. Later in year 1966, Guha and Maheswari (University of Delhi, Delhi), discovered the formation of pollen embryos from cultured anthers of wild *Datura innoxia*. However, it was not until the early 1970's that the concept of using somatic embryos began to be presented as a potential propagation system for seed-sown crops. Toshio Murashige gave a number of seminars on tissue culture propagation where he concluded with this concept. After a long period of research in his laboratory that was focused on the developmental physiology of somatic embryos which he felt to be the limiting factor for large scale propagation. Then he presented his ideas on artificial seeds at the symposium on tissue culture for Horticultural purposes in Ghent, Belgium, September 6-9, 1977. The cloning method must be extremely rapid, capable of generating several million plants daily and competitive economically with the seed method (Murashige, 1978). In mid 1970's, two separate research groups began to work on somatic embryogenesis for crop propagation Keith Walker, directed a group of scientists to identify the basic concepts of delivery of cloned, agricultural crops. Since the focus was to develop thirty somatic embryo systems that would recapitulate zygotic embryogenesis, their choice was the advanced system developed for *Medicago sativa* L. (alfalfa) using a line (Regen) identified by Bingham *et al* (1975). Soybean and vegetable crops were also of interest to them. After that, Walker cited two reports that had a strong impact on their thinking about the use of somatic embryos for crop propagation. Early in 1980, many moved to plant Genetics, Inc. where Redenbaugh *et al* (1986), discovered that hydrogels such as sodium alginate could be used to produce single embryo artificial seeds. In a few experiments, the artificial seeds were planted in the green house for plant production (7% for alfalfa and 10% for celery). According to the concepts of Street and Withers (1973), morphogenesis competence is determined from the time of culture initiation, such that there is a need to have an initiation medium that will ensure that the competent cells are involved in callus formation and also demonstrated the production of hundreds of morphologically uniform embryos from the pollens of Datura and Nicotiana. Drew (1979), developed methods to commercially propagate crops using somatic embryos. He suggested delivering carrot somatic embryos in a fluid drilling system, but was able to produce only three plants from carrot embryos on a carbohydrate free medium. He could not get success in providing many plants through this system. He faced a

crucial problem and found very slow rate of development of plantlets derived from culture and also found some coated clumps of carrot callus, embryos and roots with polyoxyethylene. Some embryos survived as well as died in desiccation step (Kitto and Janick, 1985a. The early assessments of Murashige and Skoog (1962) on the difficulty of somatic embryogeny are still valid today. The quality of somatic embryos is the limiting factors for development and scaling up of artificial seeds.

III. Applications of Gelling Agents and Plant Growth Regulators

The synthetic seeds are surrounded by a protective coating, which provides necessary protection during storage, handling and transportation. The coating could incorporate plant nutrients or plant growth regulators that aid its conversion into plantlet. In this paper, it focuses on the concepts of the axillary buds from the *in vitro* cultures which were encapsulated using sodium alginate (2.5, 3, 4 and 5% m/v) in modified MS medium and calcium chloride (50, 75, 100 and 200 mM). The beads were formed by the exchange of ions, Na ions of sodium alginate being exchanged with Ca ions forming calcium alginate. Encapsulation hardiness is determined by the optimal ion exchange of Na and Ca ions, which may vary with propagules as well as with plant species (Rai *et al* 2008). After giving 20 minutes of complexing time, the beads (artificial seeds) formed were cultured on the MS medium with BA at the rate of 1.5 mg/L and IAA at the rate of 1.0 mg/L, that gave the best axillary shoot proliferation. All the sixteen encapsulation treatments showed 100% survival and regeneration. There are several potential uses of synthetic seeds of several crop plants that are vegetatively propagated and have long juvenile periods such as citrus, grapes, mango, etc. The planting efficiency of such crops could theoretically be increased by the use of synthetic seeds instead of cuttings. A recent approach on *invitro* micropropagation and production of synthetic seeds were initiated in an endangered species of *Ceropegia barnesii*. Based on the reports, it was found that fabrication of synthetic seeds was done by encapsulating the nodal segments obtained from microshoots with sodium alginate and the most favorable medium combination for the induction of multiple shoots from synthetic seeds was MS medium complemented with 4 mg L^{-1} benzyl adenine and 1 mg L^{-1} gibberelic acid. Further, in terms of storage of synthetic seeds of *C. barnesii* it was found that the optimal temperature for storage was found to be 4°C (Ananthan *et al* 2018).

IV. Effect of Plant Propagules on Varying Concentrations of Gelling Agents

The conclusions reported by Soneji *et al* (2002) were that the concentration of 3% sodium alginate was most effective for shoot encapsulation in *Annanas cosmosus.* Encapsulated buds excised from *in vitro* proliferated shoots of MM106 apple rootstock, buds and somatic embryos encapsulation seems to be one of the promising methods for sowing buds and embryos, because encapsulation with the proper materials and structure will not only protect buds and embryos from physical damage or desiccation during the delivery or sowing process in the greenhouse, but also enable easy handling and automation (Farahani *et al* 2015). Rederbangh *et al* (1993) reported that among all the encapsulating materials, alginate hydrogel was selected as an encapsulating matrix for synthetic seeds preparation due to its moderate viscosity, low spinability of the solution, low toxicity and quick gelation which is an essential characteristic for the application of droplet hardening method. In cauliflower, the micro shoots derived from multiple shoots induction were used for the preparation of synthetic seeds. Micro shoots excised from multiple shoot cultures induced by treatment of 0.1 mg/L NAA and 5 mg/L BAP were encapsulated in 4% (w/v) sodium alginate in MS medium with 100 mM $CaCl_2$. $2H_2O$ as complexing

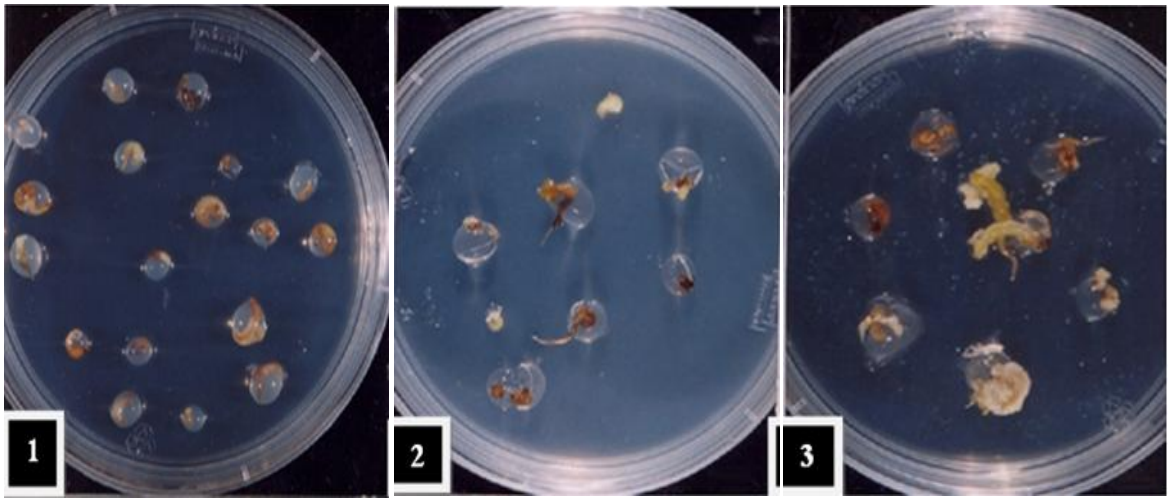
agent. In order to enhance the germination rate of synthetic seeds, the encapsulation matrix was supplemented with 0.3 mg/L NAA and 3.0 mg/L BAP in addition to the encapsulation matrix with only MS basal. This combination of PGRs was selected because it can efficiently induce both shoot and root formations simultaneously. The beads formed by the above described procedure were firm, radial and isodiametric in shape. The beads formed were 8 mm in diameter with a spherical shape. These synthetic seeds were further inoculated onto MS basal media for enhancing germination. Similarly, Nair and Raghunath (2007, 2009) further reported that for synthetic seed production in *Clitoria ternatea* and *Indigofera tinctoria*, same concentrations of sodium alginate and calcium chloride were used. In plant species such as *Morus alba* and *Rabdosia rubescens*, Padro *et al* (2012) and Ai *et al* (2012) used sodium alginate 3% solution and $CaCl_2$ 100 mM for effective bead formation. When synthetic seeds were supplemented with an encapsulation matrix such as 0.3 mg/L NAA and 3.0 mg/L BAP respectively, it showed higher germination rates, i.e. 70% and 63.33% after being stored for 7 days and 30 days. Poon Kok Siong *et al* (2011) reported that for efficient mass propagation with elite genotype in cauliflower, synthetic seed has been proved to be a potential tool which also provides an alternative method to regenerate cauliflower in transgenic studies. The reports stated by Mohanraj *et al* (2009), confirms that the presence of high percentage of sodium alginate results in the formation of beads which have a low conversion frequency. Similar reports were also concluded by Castillo *et al* (1998) in papaya who confirmed that uniform beads were formed at a lower concentration of sodium alginate added medium that is 2.5%. Encapsulated somatic embryos may survive for a period of 4 weeks stored at 4 degree celsius with a high conversion capacity (Lulsdorf *et al* 1993). Micheli *et al* (1998), reported that when encapsulated somatic embryos of olive were stored at 2 or 4 degree celsius for 23 months, showed high conversion rates of 61%. Similar results were also observed in olive and apple rootstock by Sicurani *et al* (2001), who reported that the synthetic seeds which were stored at 4 degree celsius showed a higher resistance to storage than the naked embryos and showed maximum conversion capacity since an increase in the temperature results in dessication of embryos and decrease in the conversion rate. The main reason in successful production of synthetic seeds and capsule quality is to use the correct composition of sodium alginate and cacium chloride. For production of synthetic seeds in *Minneola tangelo*, shoot tips were excised from *in vitro* proliferated shoots and encapsulated with an alginate matrix consisting of 4% sodium alginate and 100 mM calcium chloride which were found to be suitable for the formation of firm and isomatric alginate beads as well as conversion of shoot tips into the complete plantlets (Gholami *et al* 2018). Reports of Al Taha *et al* (2012) concluded that for the formation of somatic embryogenesis and plantlet regeneration from nucellus tissues of *Citrus sinensis*, somatic embryos were developed on MS medium containing BAP. Molle *et al* (1993) observed that for the production of synthetic seeds of carrot, 1% sodium alginate solution, 50 mM Calcium chloride, for duration of 20–30 min time period were found to be beneficial for proper hardening of calcium alginate capsules. It was also been suggested for the use of a dual nozzle pipette in which the embryos flow through the inner pipette and the alginate solution through the outer pipette. As a result, the embryos are positioned in the centre of the beads for better protection. The techniques of hydrogel encapsulation are also encouraged for synthetic seed production from these micro propagules. In some tropical fruits such as *Averrhoa carambola* (starfruit), *Carica papaya* (papaya), *Psidium guajava* (guava), and *Passiflora edulis* (passion fruit), as well as other orthodox seed crops, a dessicated system has been considered to be more advantageous specifically if desiccation improves maturation of embryo and also shows a high degree of tolerance to desiccation (Teng and Hor, 1977). For production of synthetic seeds,

utilization of somatic embryos for encapsulation technology was reported in various horticultural crop species by several researchers such as *Mangifera indica* L. Mango cv. Amrapali (Ara *et al*, 1999), *Psidium guajava* (Guava) (Grey and Purohit, 1991) and *Vitis vinifera* (Grape) (Rao *et al* 1996). On the basis of conclusions reported by Ganapathi *et al* (1992), for preparation of synthetic seeds in banana cv. Basrai, shoot tips were excised from the shoot cultures of banana and encapsulated in 3% sodium alginate solution prepared either in distilled water or MS medium with a combination of 0.1% activated charcoal and an antibiotic mixture. Ganapathi *et al* (2001) also reported that a conversion rate of 66% of Musa spp., artificial seed was produced by the encapsulation of their somatic embryos. They have also 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. Based on the comparison between suckers and shoot tips, Rao *et al* (1993) reported that encapsulated shoot tips were considered to be inexpensive, easier and safer material for germplasm exchange as well as for maintenance and transportation. Piccioni and Standardi (1995), observed that encapsulated micropropagated buds of six woody species such as apple (Malus spp.), blackberry (Rubus spp.), birch (*Betula pendula*), kiwifruit (*Actinidia deliciosa*), raspberry (*Rubus idaeus* L.), and hawthorn (*Crataegus oxyacantha*) were successfully regrown after encapsulating and cultivating on enriched media. In case of artificially encapsulated apical buds and axillary buds of 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) (Capuano *et al* 1998). In *Mangifera indica* L., the germination percentage of encapsulated somatic embryos was higher as compared to non encapsulated somatic embryos when inoculated in the same medium (Ara *et al* 1999). In several tree species like *Santalum album*, *Pistacia vera* and *Mangifera indica*, the somatic embryos have been encapsulated to produce synthetic seeds as reported by several researchers such as Onay *et al* (1996), Bapat *et al* (1992), etc. Gray and Purohit in 1991 mentioned that synthetic seeds have been found highly advantageous for germplasm conservation in grape and other similar crops. Similarly, Gona and Omid in 2008 observed that for conversion of fully developed plants, somatic embryos could be successfully used which has been proved to be highly beneficial for genetic transformation as well as for production of artificial seeds in strawberry. Nodal segments were more appropriate than the shoot tips for *in vitro* multiplication of plantlets of *Citrus jambhiri* Lush. Synthetic seeds were prepared using 2.5% sodium alginate dropping into 3.0% CaCl2 solution. Maximum germination was recorded when beaded shoot tips were cultured on MS medium fortified with 1 and 2 mg/L of BAP (96.67 and 100.00%, respectively) (Sharma and Roy, 2020).

V. CONCLUSIONS

In order to improve the capacity of artificial seed cultivation under non sterilized conditions, more numbers of investigations are required which could be improved by the use of accurate concentrations of anti disease and antibiotics and further detailed research is needed for improvement in the artificial seed cryopreservation capacity in several horticultural plant species. However, apart from the benefits of artificial seeds, it has been focussed that further research is required to continue in order to improve root formation of non-embryogenic artificial seeds. This technique has greater advantages such as a cheapest delivery system, minimizes cost of plantlets, offers tremendous potential in micro propagation, a promising technique for the direct use of artificial seedlings *in vivo* i.e. germplasm conservation through cryopreservation. On the other hand, this manuscript

reviews that further studies could be carried on for testing genetic fidelity in order to study the variation in germplasm during storage condition for different time period at a varied temperature.



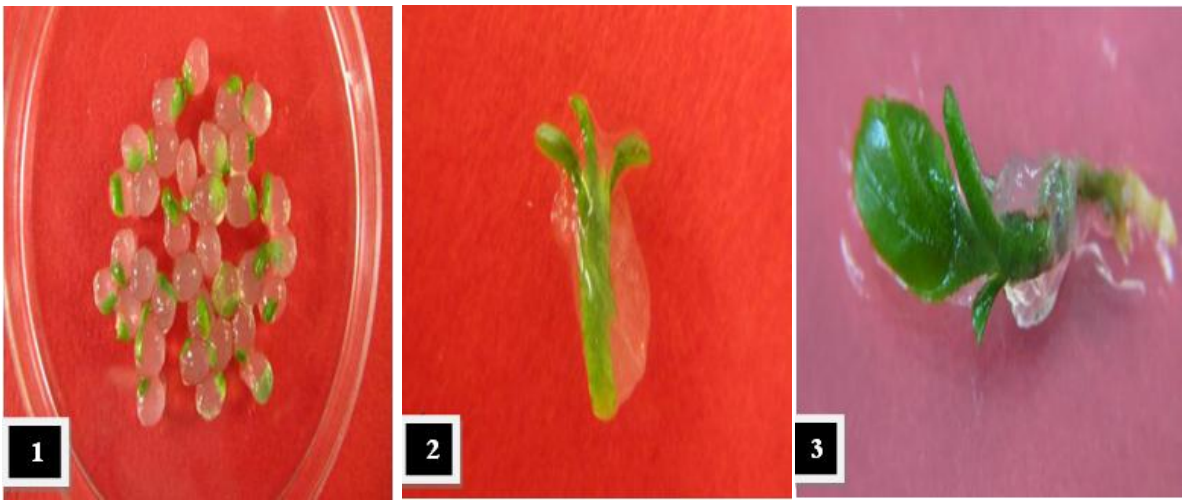
(1) Encapsulated Somatic embryos of *Litchi chinensis* Sonn.

(2) Germination of encapsulated somatic embryos showing root emergence.

(3) Emergence of shoot meristem at the tip of elongated somatic embryo.

Source: Das *et al* (2016).

Fig. 1.



(1) Encapsulation of shoot tips in 4% sodium alginate and 100 mM calcium chloride.

(2) Emergence of shoots from encapsulated shoot tips.

(3) Induction of roots in MS medium supplemented with 5 mg /L IBA.

Source: Gholami *et al* (2016).

Fig. 2. Various stages of regeneration of plantlets from encapsulated shoot tips of *Thamson navel*.

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