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Synthetic Seed – Future Prospects in Crop Improvement

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Abstract **– Ripened ovule containing embryo has the capability to regenerate is called seed, the vehicle that connects plant kingdom from one generation to another. Syn seeds are defined as artificially encapsulated somatic embryos, shoot buds, cell aggregates, or any other tissue that can be used for sowing as a seed and that possess the ability to convert into a plant under in vitro or ex vitro conditions and that retain this potential also after storage. Artificial seed production technique is method for mass propagation of promising plant genotypes and also alternate technology of propagation in many commercially important crops. The production of plant clones multiplied by tissue culture and distributed as artificial seeds could compensate the costly F_1 hybrids for different plant crops. The delivery of artificial seeds facilitates scaling up in vitro cultures and acclimatization to ex vitro conditions. The development of an artificial seed technique provides a great approach for the improvement of various plant species such as trees and recalcitrant seed crops. The principle limitation for commercialization has been the somatic embryogenesis. Research on encapsulation of propagules should be taken up. This review paper dealt with concept, types of syn seed, methods of production and its application in crop improvement.**

Keywords **– Synthetic Seed, Somatic Embryo, Encapsulation, Micro Propagation, Syn Seed.**

I. INTRODUCTION

The Natural Seed

The seed stage of any plant shows a unique developmental phase of the spermatophyte life cycle. Anatomically a seed consists of parental sporophyte tissue viz. the seed coats, which are derived from the integuments and nucleus: some endosperm (contain variety of stored materials such as starch, oils, proteins etc.), which may either gametophytic tissue or fertilized tissue: and the embryo, the new young sporophyte.

Concepts of Tissue Culture and Artificial Seeds

P.R. White is father of tissue culture in the United States and was first to grow excised root tips of tomatoes in continuous culture. The plants which are vegetatively propagated are called as clone having similar genetic makeup as that of single parent. The liquid cell suspension cultures have significance for mass production of cells from friable callus which can form embroids (somatic embryos, fig 1) in the process of embryogenesis.

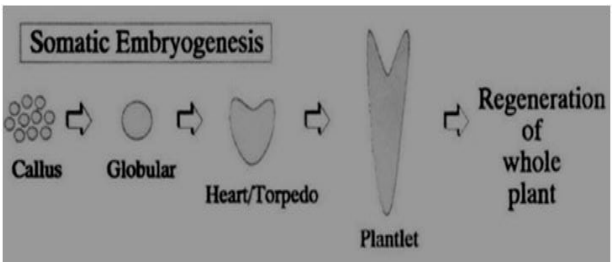

Fig. 1. Different stage of somatic embryo development (Zimmerman, 1993).

Suspension culture can be modified by continuous introduction of fresh medium in to the suspension culture, enabling production of thousands of embryos in single container with minimal manual transfer.

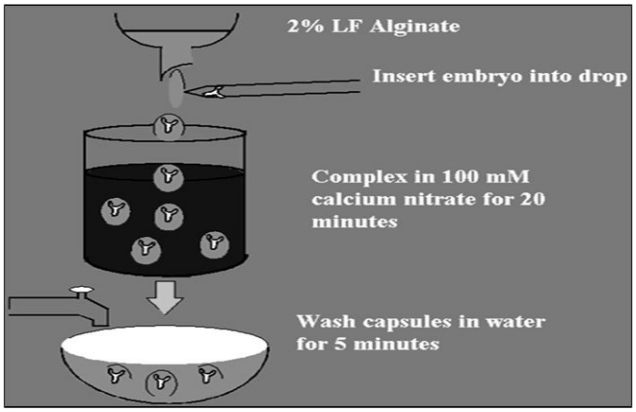

Fig. 2. Suspension culture method for production of artificial seed (modified from Redembaugh et al.1991)

Somatic embryos or shoot buds or meristem tissue or any totipotant cells which is artificially encapsulated by chemicals. Seeds are encapsulated by protective gel like sodium alginate gel (0.5–5.0% w/v) and dropped into a calcium salt solution $CaCl_2$ (30–100 mm), Ca $(NO_3)_2$ (30–100 mm)].

The artificial seed structure mimics that of the conventional seed. It consists of both explants 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 (Cates *et al*, 2009). At the biotechnology division of BARC, research on the development of protocols for synthetic seeds using somatic embryos, axillary buds and shoot tips is in progress in five economically important plants, sandalwood, rice, mulberry, banana and cardamom.

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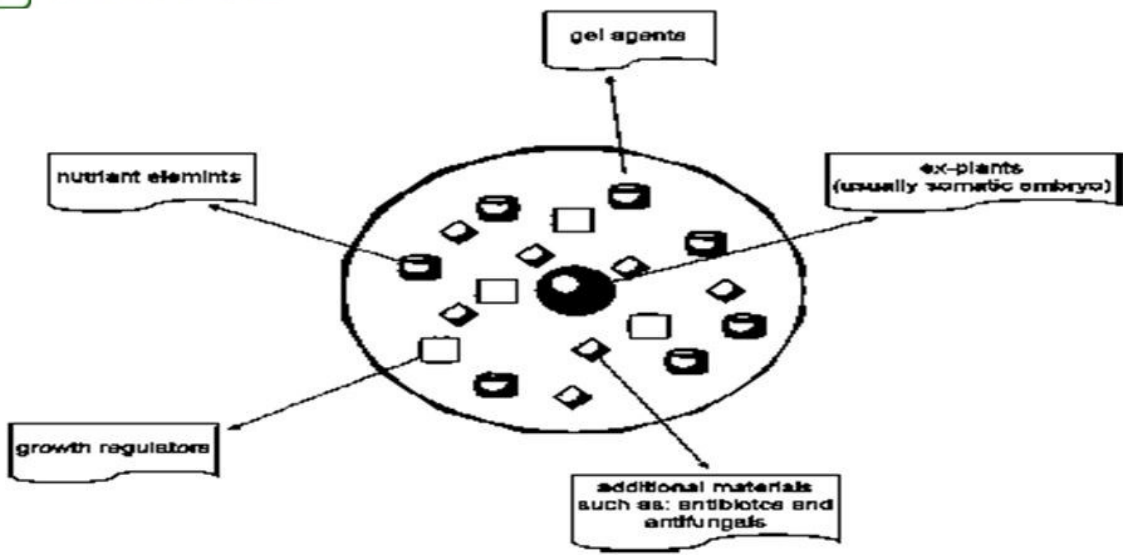

Fig. 3. Artificial seed concept

Discovery of Synthetic Seed

The origin of the idea of an artificial seed is difficult to determine. **Steward et al., 1958 and Reinnert, 1958** was first who produced somatic embryos and procedure of somatic embryogenesis in carrot. The first time the idea of synthetic seed was given by **T. Murashige (1977).** He conducted 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. He presented his ideas on artificial seeds at the Symposium on the Tissue Culture for Horticultural purposes in Ghent, Belgium, Sep 6-9, 1977. He commented in proceedings that *the cloning method must be extremely rapid, capable of generating several million plants daily and competitive economically with the seed method*. During mid 1970's, **Keith Walker** identified basic concepts of delivery of cloned, agricultural crops to develop somatic embryo system using a line (Regen S) identified by **Bingham *et al.* (1975)** in alfalfa. **Redenbaugh *et al.* (1984)** developed a technique for hydrogel encapsulation of individual somatic embryos of alfalfa. **Street (1977)** advocated the problem of reliability in embryogenesis according to which morphogenic competence will ensure that the competent cells are involved in callus formation. **Sunderland (1977)** demonstrated that the production of hundreds of morphologically uniform embryos from *Datura* and *Nicotiana* pollen. **Robert Lawrence (1981)** started to develop various methods for cloning of forest tress and also focused on delivery of somatic embryos using fluid drilling technology and using polyoxyethylene to form seed tapes or sheets. In one symposium workshop Lawrence and walkers group introduced with each other and discussed about how low-cost, high-volume propagation system can be developed for vegetables and agronomic crop using somatic embryo and delivered by fluid drilling. **Drew (1979)** developed methods for commercially propagate crop using somatic embryos. **Murashige and Street (1977)** suggested that quality and fidelity of somatic embryos are limiting factors for development and scale-up of artificial seeds. **Kitto (1982)** and coworkers prepared first synthetic embryos in carrot. **Bapat *et al.* (1987)** proposed the encapsulation of shoot tip in *Morus indica.* **P.S. Rao's group** from BARC, Trombay reported artificial seeds prepared from shoot buds for plant propagation. P.S. Rao and his associates have reported high frequency somatic embryogenesis from *Indica* rice cultivars (**Suprasanna *et al.*, 1995**) and utilized for artificial seed production. Plant species *Brassica campestris* **(Kitto and Janick, 1985),** *Mangifera indica L. Mango cv. Amrapali* **(Ara et al, 1999),** *Psidium guajava (Guava)* **(Grey and Purohit, 1991)**, *Solanum melongena (Eggplant)* **(Akhtar, 1999)** and *Vitis vinifera (Grape)* **(Rao et al.1991)** in which somatic embryo used for encapsulation technology to produce synthetic seeds. Non zygotic explant tissue can be used in somatic embryogenesis in coffee.

Artificial Seed

Artificially encapsulated somatic embryos shoot buds, cell aggregates, or any other tissue that can be used for sowing as a seed and that possess the ability to convert into a plant. The preparation of seed analogues called synthetic seeds or artificial seeds from the micro-propagules like somatic embryos, auxillary shoot buds, apical shoot tips, embryogenic calli as well as protocorm or protocorm like bodies. It is living seed-like structure derived from somatic embryoids *in vitro* culture after encapsulation by a hydrogel.

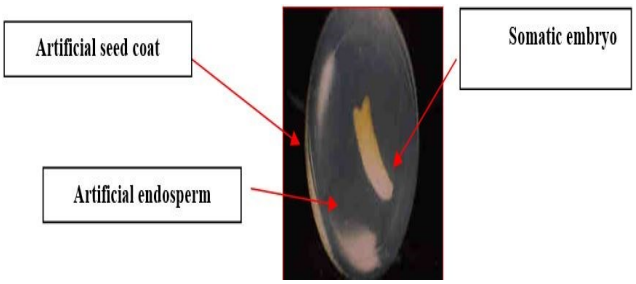

Fig. 4. Structure of Syn seed

Table I. Comparison between natural and artificial seed

SYNTHETIC SEEDS	*NATURAL SEEDS*
Produced from the asexual process	*Produced from the sexual process*
Doesn't involve the fusion of Gametes	*Involve the fusion of male and female Gametes*
Produced from Vegetative Cells	*Produced From Germ Cells*
Contains genetic constituent from single parent	*Contains genetic constituent from both parents*
Genetic recombination does not take place	*Genetic recombination takes place*
Contains only embryo and endosperm and seed coat are absent	*Contains embryo, endosperm and seed coat*

Need of artificial seed

Artificial seed is needed as most of recalcitrant seed cannot be preserved in slow growth cultures or under cryopreservation, can be only propagated as synthetic seed and many fruit crops are difficult to multiply by conventional propagation methods. Seed propagated hybrids can be multiplied through tissue culture and propagated by using synthetic seeds rapidly.

Basic and Essential Requirements for the Production of Artificial Seeds

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. Plant lines which are produced via somatic embryos are capable of keeping their regenerative capacity for a long time, resulting in uniform plant production because it avoids the dedifferentiation callus stage, and consistent genetic structure production. The production of artificial seeds via somatic embryos have been investigated in several plant species including carrot (*Daucus carota*), alfalfa (*Medicago sativa*), Norway spruce (*Picea abies*), sandalwood, pistachio (*Pistacia vera*), sandalwood (*Solanium album*), grape (*Vitis vinifers*) and mango (*Mangifera indica* L.), *Citrus reticulata*, *Hopea parvitflora, Paulownia elongata*, sugarcane, *Oryza sativa* (hybrid rice), Rotula aquatic (takad), *Daucus carota* (Latif et al., 2007), *Pinus radiata, Nothofagus alpina*, *Catharanthus roseus* (L.).

The conversion level of carrot embryos can be raised from 0% to 53–80% by applying three essential treatments: (i) culturing the embryos (ii) dehydration of embryos and (iii) post-dehydration culture on nutrient medium (Onishi *et al.*1994*)*. (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} GA3, 0.01 mg_L^{-1} BAP and 2% sorbitol. 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.

Two types of artificial seeds (encapsulated somatic embryos) are commonly produced: desiccated and hydrated (Bapat, *et al*., 2005).

1. *Desiccated Artificial Seeds*

Desiccated artificial seeds are 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. The desiccation tolerance can be induced using a high osmotic potential of the maturation medium and also by applying sub-lethal stresses such as nutrient deprivation or low temperature. The osmotic potential could be increased by using high gel strength or by the addition of permeating osmoticants such as mannitol, sucrose, etc.

Ex: Carrot, celery embryos

2. *Hydrated Artificial Seeds*

Hydrated artificial seeds can be produced by encapsulating somatic embryos in hydrogel capsules to supply protection and to convert the in vitro micropropagules into ‘artificial seeds’ or ‘syn seeds’. They are produced in plant species which are recalcitrant and sensitive to desiccation.

Ex: Alfalfa Barley, Sandal wood

Gelling Agent and Adjuvant Materials

Agar, Alginate, Carboxy Methyl Cellulose, Sodium Pectate, etc. were tested for synthetic seed production, out of which Alginate encapsulation was found to be more suitable for production of synthetic seed. Usually 2% sodium alginate gel with a complexing solution containing 100 mM Ca^{2+} is used and is found to be satisfactory. They provide the nutrient elements and a protective layer, which makes them easier to handle and store. The major principle for alginate encapsulation formation depends on the exchange ions between Na^{+} in sodium alginate with Ca^{+} in $CaCl_2.2H_2O$, which happens when sodium alginate droplets involving the artificial embryos or any other plant propagule is dropped into the $Ca_2.2H_2O$ solution, producing stable explant beads.

Artificial Endosperm

Endosperm used as a carrier for micro-organisms, nutrients, antibiotics, plant growth regulators, pesticides and fungicides. It provides not only the physical protection for embryos but also the carbon source and growth regulators to control and sustain growth through germination.

Formulation of Endosperm:

Some of the Growth hormones, like Abscisic Acid (1 µM) and Mannitol (0.25 M). Amino Acid supplements like Proline, Glutamic Acid and Arginine are supplied.

Artificial Seeds Storage Ability

The protocol consists of three procedures:

(a) Pre-culturing encapsulated explants in a medium containing high concentrations of sucrose;
(b) Drying the encapsulated micro-organism; and
(c) Direct plunging into liquid nitrogen.

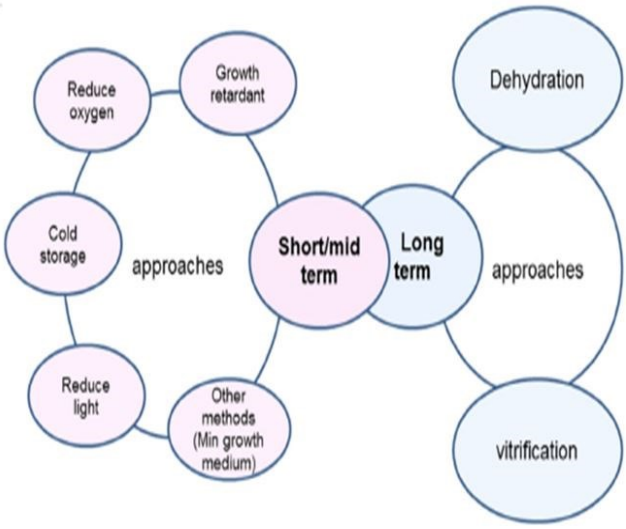

Fig. 5. Syn seed storage approaches (Mohamed, 2005).

II. Production of Synthetic Seed

The preserved embryoids without seed coat are termed as synthetic seeds. In vitro embryoid develops from callus tissue callus tissue and their induction is initiated by somatic embryogenesis supplementing the medium with auxin and cytokinins in proper ratio. Seeds are contaminated with microbes and desiccated are encapsulated by protective gel like calcium alginate.

Establish somatic embryogenesis
↓
Mature somatic embryos
↓
Synchronize and singulate somatic embryos
↓
Mass production of somatic embryos
↓
Standardization of encapsulation
↓
Standardization of artificial endosperm
↓
Mass production of synthetic seeds
↓
Greenhouse and field planting

Fig. 6. Schematic presentation of steps of synthetic seed production.

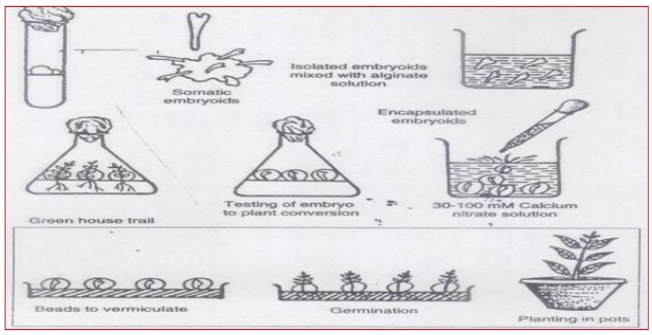

Fig. 7. Flow diagram presenting the procedure of synthetic seed production

The Following Steps are Needed for Commercial Synthetic Seed Production

1. Production of embryogenic tissue from transformed cells or tissue.
2. Large – scale production of synchronous somatic embryo.
3. Maturation of somatic embryo.
4. Non – toxic encapsulation/coating process.
5. Artificial endosperm/ mega gametophyte, depending on species.
6. Storage capability of artificial seeds.
7. High frequency, direct green house / nursery field for conversion to plant, depending on production requirements.

The Events which are Associated with the Process of Embryo to Plant Conversion

1. Germination.
2. Development of vigorous root system.
3. Development of shoot meritem.
4. Production of true leaves.
5. A direct shoot to root connection.
6. Absence of hypertrophy of the hypocotyls.
7. Minimization of callus growth in the hypocotyl.
8. A green plant with a normal phenotype.

Two Standard Methods have been used for Encapsulation of Somatic Embryos.

1. *Gel Complexation Via a Dropping Procedure*

This is the most useful encapsulation system. Drip 2-3 % sodium alginate drops from at the tip of the funnel and the somatic embryos are inserted. Keep the encapsulated embryos complex in calcium salt for 20 min. Rinsed the capsules in water and then stored in air tight container.

2. *Automate Encapsulation Process*

This is the quick method of artificial seed production. Alginate solution with embryo is feed from supply tank. Alginate capsules were planted in speeding trays using a vacuum seeder. The capsules are planted in the field using a stan hay planter. A hydrophobic coating is required for mechanical handling for the rapid drying and the thickness of the alginate capsules. For coating, an Elvax 4260 copolymer is suitable for producing a slow drying, non tacky coating which allows embryo conversion.

Mass Balance Concept

Mass balance is amount of tissue at the beginning of the experiment and the number of high quality plants produced at the end. The artificial seed package,

consisting of a calcium alginate bead coated with a hydrophobic Elvax polymer.

III. Advantages of Synthetic Seed

1. Direct delivery of somatic embryos will save many subcultures to obtain plantlets from regenerated embryo.
2. Reduced costs of transplants.
3. Direct greenhouse and field of delivery of elite, select genotypes, hand-pollinated hybrids, genetically engineered plants, sterile and unstable genotypes, large–scale monocultures, mixed genotype plantation.
4. Carrier of adjuvants such as plant growth regulators and pesticide protection of meiotically – unstable, elite genotypes.
5. Comparative aid for zygotic embryogeny.
6. Production of large numbers of identical embryo.
7. Determine role of endosperm in embryo development and germination.
8. Study of seed coat formation.
9. Synthetic seeds are true breed.
10. Potential advantages of artificial seed technology for tree genetic engineering.
11. The encapsulation of somatic embryos provides a potential method to combine the advantage of clonal.
12. Propagation with low cost, high volume capabilities of seed propagation.
13. Seeds are produced within a short time.
14. Seeds can be produced at any time and in any season of a year.
15. Dormancy of artificial seed can be shortened by reducing the life cycle of plant.
16. These are useful in germplasm preservation.
17. Syn seeds are applicable for large scale mono culturing.
18. Seeds give protection of meiotically unstable, elite genotype.
19. The synthetic seeds provide us knowledge to understand the development, anatomical characteristics of endosperm and seed coat formation.

IV. Disadvantages of Synthetic Seed

The major challenges that need to be solved to improve the protocols are in storage caused by lack of dormancy, synchrony in somatic embryo development, improper maturation, slow conversion into plantlets, low production of viable mature somatic embryos, proper acclimatization into field because of their tenderness due to the absence of lignification and low cuticle formation and the reduction in viability and plant recovery when the artificial seeds are stored at low temperature can be overcome if the process and regulation of somatic embryogenesis and origin of somaclonal variation. In recalcitrant species, somatic embryogenesis, the possibility of using non-embryogenic propagules for artificial seed production was reported to be a promising such as the difficulties of achieving one rooting step. The difficulties of sowing artificial seeds directly in soil or under non-sterile conditions are considered to be one of the main limitations of this technique.

V. Conclusion

The technique has great advantages such as: a cheapest delivery system, minimize 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. However, despite the advantages of artificial seeds, further research is required in order to improve root formation of non-embryogenic artificial seeds. The further detailed research is needed for improvement in the artificial seed cryopreservation capacity in some plant species.

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