

## Prospects and Limitations of Synthetic Seeds

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### ABSTRACT

*Synthetic seed technology is one of the most important tools to breeders and scientists of plant tissue culture. It provides a powerful advantage of rapid and large scale multiplication, minimum labour, and low cost propagation of seed less, hybrid, and many vegetatively propagated readily infected plants. This technology is useful in multiplying transgenic plants, somatic and cytoplasmic hybrids, sterile and unstable genotypes and also for cryopreservation of desirable elite genotype. It is not successful in some of the horticultural crops seeds propagation as; some seeds are minute in size and heterozygosity of seeds particularly in most of the cross pollinated crops, eg; orchids, presence of minimized endosperm, some seeds essential mycorrhizal fungi association for germination eg: orchids, no seeds are formed. These crop species can be multiplied vegetatively by producing synthetic seeds. These can be somatic embryos, enfolded cell aggregates, shoot buds, or any other type of tissue that may be effectiveness for planting materials or that has capability to develop into a plant under in situ or ex situ conditions and that can retain storage potentiality (Capuano et al., 1998). An embryo produced by somatic embryogenesis is kept in a synthetic seed that has an artificial seed covering, artificial medium that provides nutrition, and an artificial seed. The term "synthetic seed" was first used in 1977 by Toshio Murashige. The first artificial seeds were created in 1982 by Kitto and Janick using somatic carrot embryos. This method is necessary to reproduce hybrids because plants cannot multiply or produce seeds. It also allows for the production of male or female sterile plants, elite genotypes of disease-free plants, conservation of recalcitrant species, transgenic plants, etc.*

**Keywords:** *Seeds, embryos, sterile plants*

### INTRODUCTION

These days, one of the most vital resources for plant tissue culture scientists and breeders is artificial or synthetic seed technology. It offers the significant benefits of large-scale, quickly multiplying, low labor, and inexpensive propagation of many vegetatively propagated, hybrid, and easily infected plants without the need for seeds. This technology is useful in multiplying transgenic plants, somatic and cytoplasmic hybrids, sterile and unstable genotypes and also for cryopreservation of desirable elite genotype.[1]

### SYNTHETIC SEED

- Synthetic or Artificial seeds that are encased in cell aggregates, shoot buds, somatic embryos, or any other tissue that could be useful as planting material or that has capability to develop into a plant under *in situ* or *ex situ* conditions and that can retain storage potentiality (Capuano et al., 1998) as shown in Figure 1.
- In simple words synthetic seed contains an embryo produced by somatic embryogenesis enclosed within an artificial medium that

supplies nutrients and is encased in an artificial seed covering.

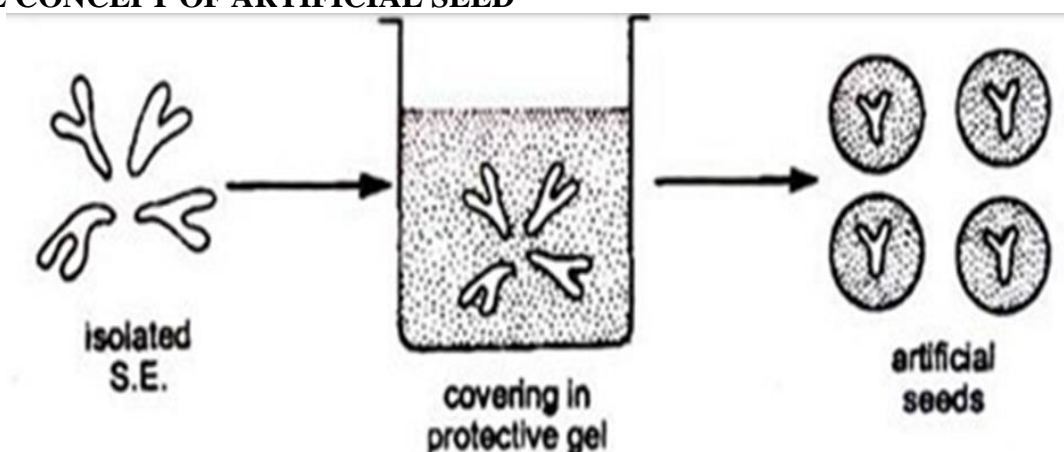
- Toshio Murashige in 1977 first coined the term synthetic seed
- Carrot somatic embryos were used by Kitto and Janick in 1982 to create the first synthetic seeds.

#### IMPORTANCE[2]

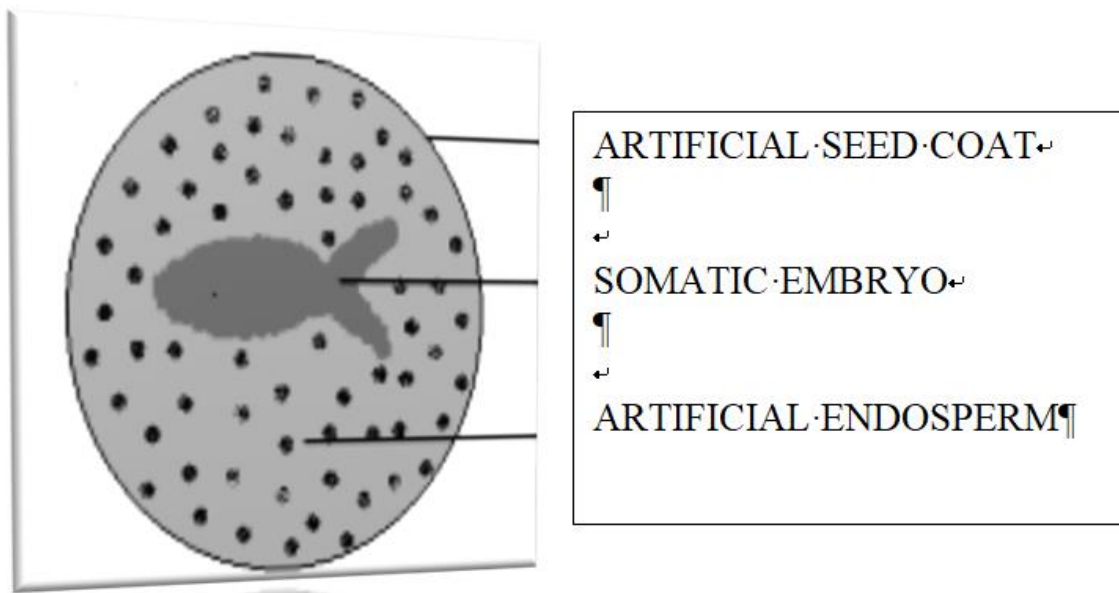
- Propagation hybrids, plants which can't produce seed, or multiplication elite cultivars high value rare plants.
- Propagation of sterile plants, either male or female.
- Recalcitrant seed germplasm conservation.
- Transgenic plants conservation.
- Disease free plants production



#### THE CONCEPT OF ARTIFICIAL SEED

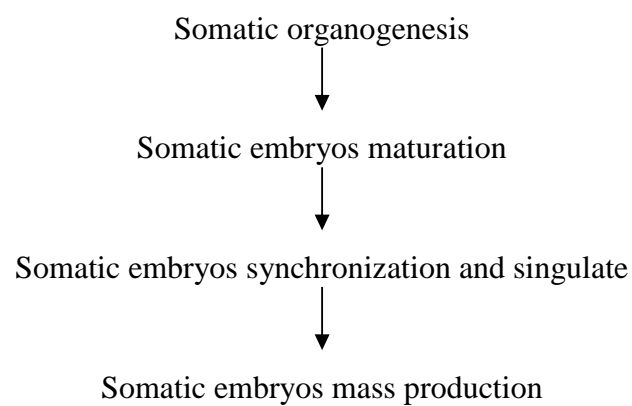


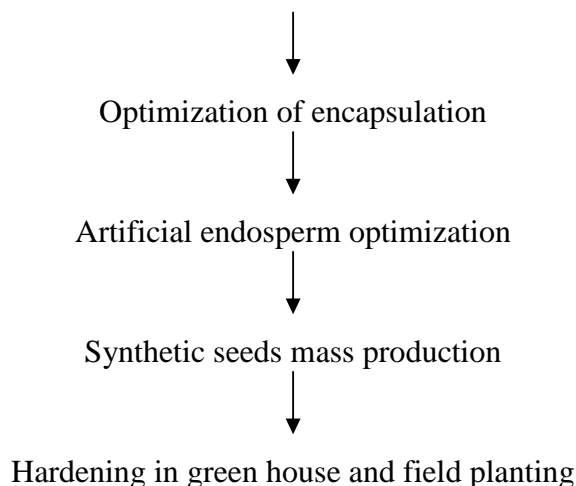
*Fig. 1: Outline of preparation of synthetic seeds.*



### SYNTHETIC SEEDS PRODUCTION[3]

#### Steps





#### Basic Necessity

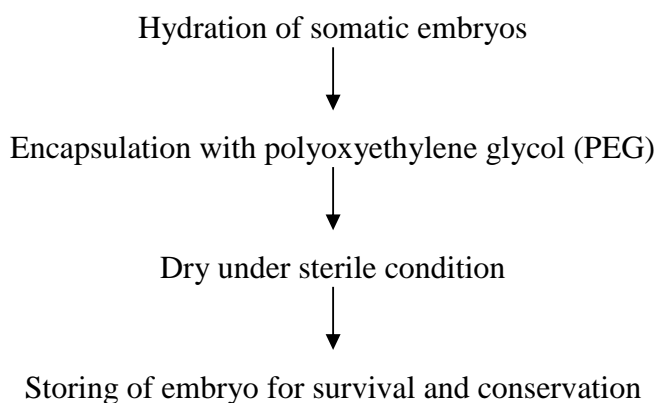
- Healthy somatic embryo, root tip or shoot tip
- Encapsulation and coating systems
- Adjuvents like hormone, nutrients etc.

The somatic embryos used to create the desiccated synthetic seeds are either naked or encased in polyoxyethylene glycol (Polyoxr), after which they are dried out as shown in Figure 2. These kinds of artificial seeds are only made by plant species whose somatic embryos can withstand desiccation.

#### Synthetic Seed Production Methods can be of Two Types[4]

##### Desiccated synthetic seeds

#### Steps



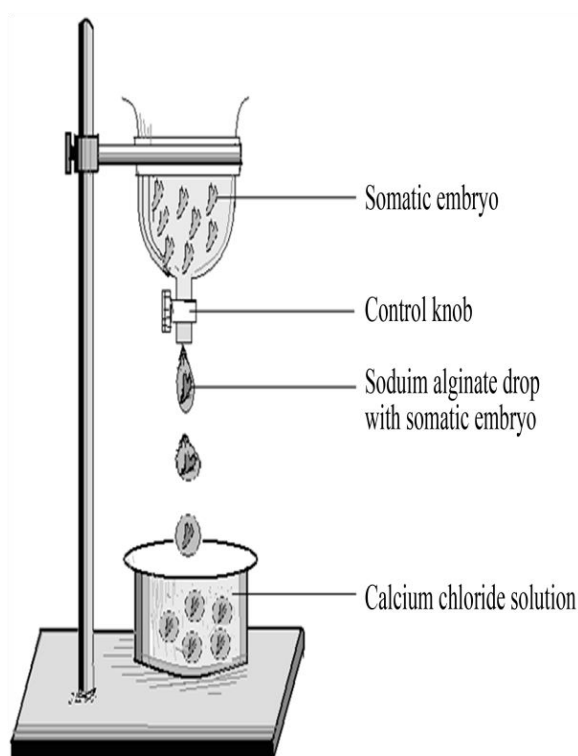
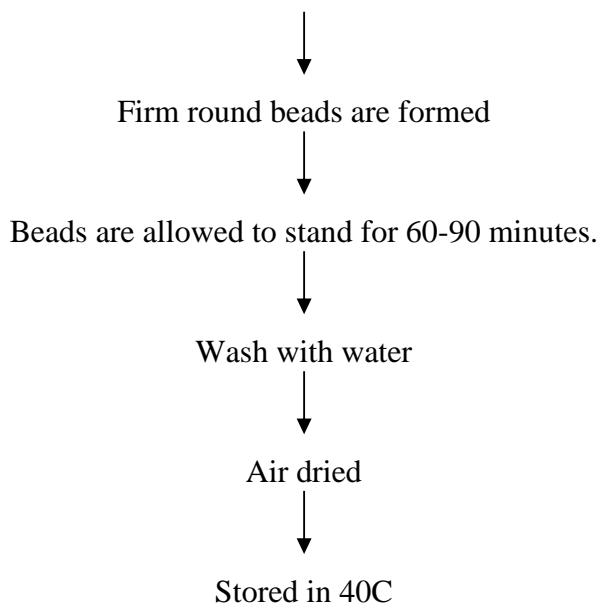
#### Hydrated synthetic seeds [5]

- In plant species where the somatic embryos are resistant to desiccation and recalcitrant, hydrated synthetic seeds are generated.
- These are prepared by condense the somatic embryos in hydrogels like sodium alginate, sodium alginate with gelatin, potassium alginate, sodium pectate or carrageenan.

#### Hydrated System

Encapsulation of somatic embryos in hydrogel like sodium alignate

Dropping in calcium chloride solution



**Fig. 2:** Digrammatic representation of semi automated encapsulation of somatic embryos.

### SOMATIC EMBRYO

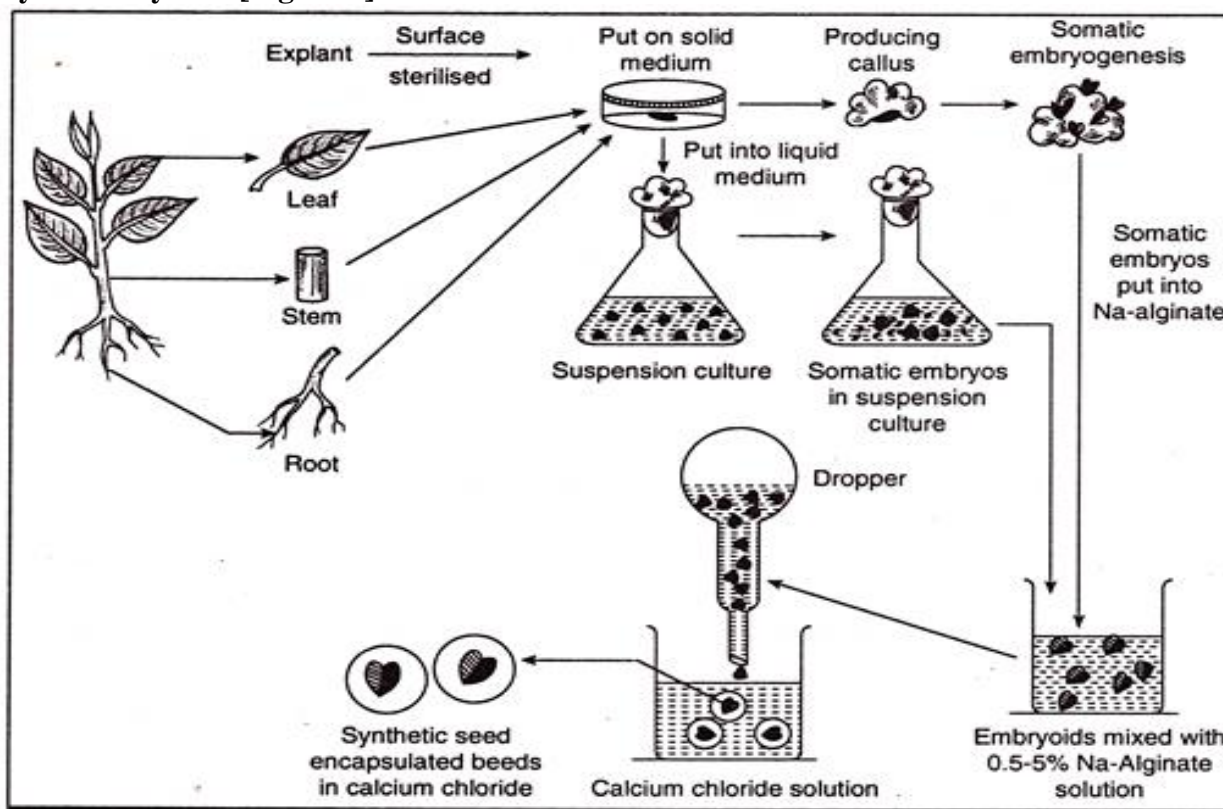
It is undulating formation with both basal and apical meristematic site, which are able to produce root and shoot

respectively. It is sometimes referred to as an 'embling' from a plant derived from a somatic embryo. [6]





**Hydrated System [Figure 3]**



**Fig. 3: Steps for synthetic or artificial seed production.**

#### **ENCAPSULATION [Figure 4-7]**

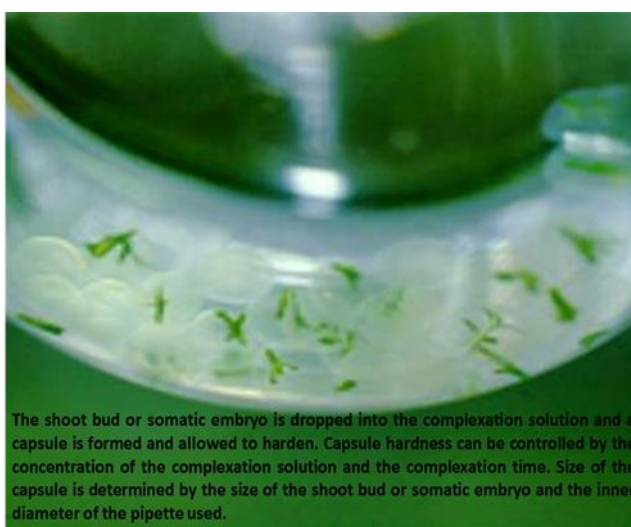
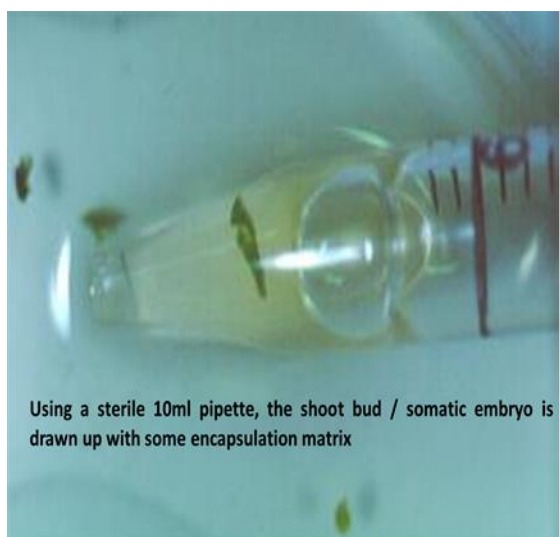
For production and to protect synthetic seeds, encapsulation is essential. It is prepared by several types of hydrogels

(water soluble). The hydrogel has complexing material which is utilized in different concentrations.

Hydrogelling agent (% w/v)	Complexing agent ( $\mu\text{M}$ )
1. Gelrite (0.25)	1. Calcium salts (30 –100)
2. Sodium alginate (0.5 – 5.0)*	2. Ammonium chloride (500)
3. Carragenan (0.2 – 0.8)	3. Calcium chloride (30 –100)
4. Sodium alginate (2.0) with Gelatin (5.0)*	4. Potassium chloride
5. Locust beam gum (0.4-1.0)	5. temperature lowered

\*Alginate hydrogel is a protecting synthetic seed from microbial infection. It is chosen for synthetic seed as it has ability

to moderate density and low flexibility, less infection for somatic embryos and speedy gelatin.



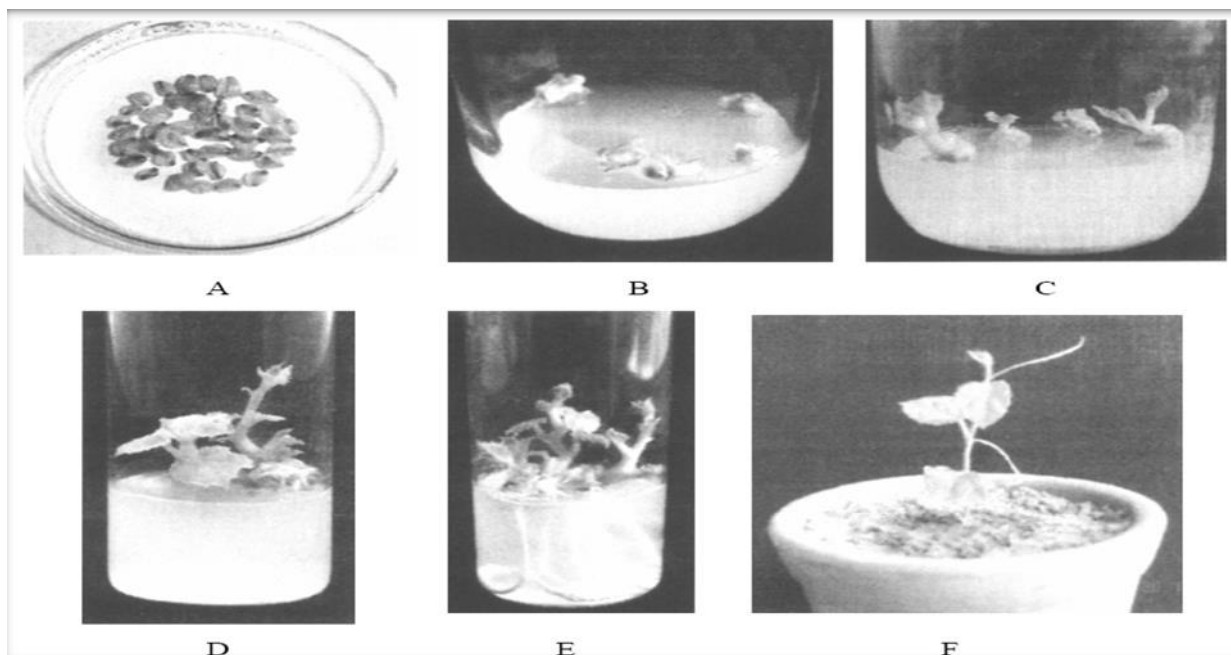
**Fig. 4:** Encapsulated synthetic seed.



*Fig. 5: Orchid synthetic seed produced by alginate encapsulation.*



*Fig. 6: Orchid plantlets from synthetic seed.*



*Fig. 7: Growth of synthetic seed (encapsulated shoot tips) of pointed gourd.*



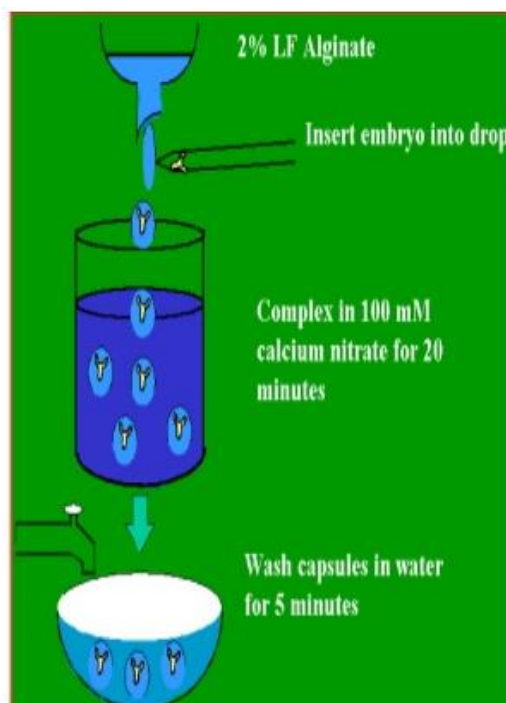
- A. Encapsulated shoot tips in sodium alginate bead.
- B. Germination of encapsulated shoot tips.
- C. Shoot induction of encapsulated shoot tips.
- D. Shoots from encapsulated shoot tips
- E. Rooted multiple shoots of encapsulated shoot tips.
- F. Established plant in earthen pot.

**TWO STANDARDIZED METHODS  
HAVE BEEN USED TO COAT  
SOMATIC EMBRYOS**

**Encapsulation Methods for Synthetic Seed**

**Dropping procedure**

- 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 capsule in water and then stored in a air tight container



**Molding**

- This technique involves mixing embryos with a temperature-dependent gel (such as agar or gel rite) in a straightforward manner.
- Gel is applied to cells as the temperature drops.

**Preparation of Artificial Endosperm and its Storage Procedure**

- When zygotic embryos are developing into seeds, somatic embryos lack the endosperm that supplies them with nutrients. Additionally, the seed coat that shields the seed is absent from these.
- For adding these insufficiencies, insertion of nutrient and growth promoters to the encapsulated matrix is required. It is also called artificial endosperm.

- For increasing in ability of synthetic seeds for germination and viability artificial endosperm have to add.
- The optimum temperature for storage of synthetic seeds is 4°C. In this temperature maintained seed viability even up to six months when stored.
- Beneficial substances such as microorganisms, fungicides, pesticides, nutrients, and antibiotics can be added to the encapsulation matrix.
- Activated charcoal can be added to upgrade the conversion and vigour of encapsulated somatic embryos.
- For increasing desiccation tolerance, different type of physical or chemical stresses can bided during somatic embryos development (Proline, ABA, Triazoles, nutrient deprivation, High sucrose concentration, chilling, high density inoculum and thermal shock).

#### Example of Crops for production of synthetic seed

<i>In vitro</i> propagating materials	Crops
Somatic embryos	Rice, wheat, triticale, maize, sorghum, horse raddish, alfaalfa, brinjal, celery, carrot, brassica, lettuce, potato, cucumber, asparagus, soyabean, coffee, sugarcane, cotton and tobacco
Shoot tips	Cardamom, banana, and <i>Carum carvi</i>
Adventitious or axillary buds	Grape, mulberry and eucalyptus

#### SOME PRACTICAL APPLICATIONS

- Synthetic seeds have been found highly advantageous for germplasm conservation in grape and other similar crops.
- Synthetic seed of apple m26 rootstock give effective result in mass production(Capuano G *et al.*,1998)
- Edible and cultivated banana cultivars are highly sterile, triploid and producing seedless fruits through parthenocarpy. The uniformity of growth of *in vitro* obtained plants makes it possible to control the flowering time and harvesting. Hwang *et al.*1984 express higher survival rate in the field, and produce a significant enhance in yield and fruit quality.
- Alginate encapsulations of axillary buds of *Ocimum sanctum*, *Ocimum basilicum*, *Ocimum americanum*, *Ocimum gratissimum* and their regrowth even after 60 days of storage at 4°C were reported by Mandal *et al.* (2000).
- Naik and Chikkagouda (1997) found that mass propagate sugarcane *in vitro*, sugarcane plantlets were regenerated from encapsulated somatic embryos derived from callus of sugarcane lines GSBT - 1. They reported that plants *in vivo* of *Saccharum officinarum* from artificial seeds were taller and had a smaller diameter at eight months, but these differences disappeared at 12 months.
- Fujii *et al.* (1998) found improvement of plantlet growth and vigor of Alfalfa through artificial seeds
- There is huge scope of synthetic seeds of forestry tree and medicinal plant like *Arnebia euchroma* as replacement planting material in near future.

#### Prospects

1. Easy handling
2. Inexpensive transport
3. long storage life
4. Product uniformity
5. Avoids extinction of endangered species

6. Large scale production
7. Germplasm conservation
8. Not a season dependent technology.
9. Permits direct field use
10. Supply of beneficial adjuvants
11. Propagation of plants unable to produce viable seeds
12. Production of hybrids

### Restrictions

Synthetic seed technology is seen a bright scope for propagating material of number of plant species. Some reasons for practical utility:

- Restricted production of fruitful micropropagules helpful the production of synthetic seed.
- Sometime somatic embryos developed abnormal and asynchronous.
- Somatic embryos mature improperly ultimately effect on germination and vigour.
- Due to absence of dormancy and stress tolerance in somatic embryos, storage period is less.
- Uniformity of synthetic seed will be loss due to somaclonal variation.

### CONCLUSIONS

Artificial or synthetic seed is a new vista in agriculture which originated from somatic embryos. It is a promising technique for multiplication of elite cultivars, transgenic crop and the crop having problems to produce true to type seed. It is a clonal propagation technique which minimizes the tough selection method of conventional recombinant breeding procedure.

This modern biotechnology can bring to the doorsteps of the farmers with cost effective way. In germplasm conservation, it can provide massive potential. More fruitful accurate research is required to refine the technology so that it can be used as commercial basis.

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