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Synthetic seed: An overview

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Abstract
In-vitro encapsulation of somatic embryos, shoot buds, or any aggregates of cells can be achieved through artificial seed production. There can be two types of artificial seed production one is - Desiccated seeds which can be produced by encapsulation in polyoxyethylene glycol or naked and another is- Hydrated seeds which can be produced by encapsulating in hydrogels like sodium alginate, potassium alginate etc. Encapsulation of seed can be achieved either through dropping or molding method. Different concentration % (w/v) of gel can be used such as sodium alginate (0.5-5.0), sodium alginate with gelatin (2.5), carrageenan (0.2-0.8),locust bean gum(0.4-1.0), gelrite (0.25) in complexing agent at concentration (µm) of calcium salts (30-100),calcium chloride(30-100),potassium chloride &ammonium chloride (500).Addition of adjuvants or various essential nutrients, pesticides can be done to achieved artificial endosperm to artificial seed. At last, cryopreservation can be done using several techniques like vitrification, controlled rate cooling, encapsulation etc.

Keywords: Synthetic seed, desiccated seeds, essential nutrients

Introduction
Artificial seeds are encapsulation of somatic embryos, shoot bud, aggregates of cell of any tissues in gel matrix under aseptic condition in an sterile environment later on which can be sown into a plant either in in-vitro or in-vivo condition. The somatic embryo can be encapsulated, handled and used like a natural seed was first suggested by Murashige (1977) [6] and efforts to engineer them into synthetic seed have been ongoing ever since (Gray, 1987) [3]. Bapat *et al*. (1987) [1] proposed the encapsulation of shoot tip in *Morus indica*; this application has made the concept of synthetic seed set free from its bonds to somatic embryos and broaden the technology to the encapsulation of various *in vitro* derived propagules. He defined artificial seeds as "an encapsulated single somatic embryo". Later on Gray *et al.* defined it as "a somatic embryo that is engineered for the practical use in commercial plant production". As artificial seed have ability to extend its period of storage they can eliminate the acclimation steps necessary in micro propagation and give breeders greater flexibility. The term, "EMBLING" is used for the plants originated from synthetic seed.

Types of artificial seeds
Desiccated artificial seeds
These type of seeds are achieved from somatic embryos 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. This type of synthetic seeds is produced in desiccation tolerant species plant such as craterostigma plantagineum, lindernia brevidens, ramonda serbica.

Hydrated synthetic seeds:
Hydrated synthetic seeds are produced by encapsulating the somatic embryos in hydrogels like sodium alginate, potassium alginate, carrageenan, sodium pectate or sodium alginate with gelatine. It is the most studied method of artificial seed production. Such seeds are produced in those plants where somatic embryos are recalcitrant and sensitive to dessication. Calcium alginate gel is most suitable and used gel.

Encapsulation methods for artificial seeds
Dropping method
Prepare 1-5% sodium alginate solution and insert somatic embryos in the prepared gel.

Then take this sodium alginate gel containing somatic embryos in the funnel and drip the drops of gel along with somatic embryos from the tip of funnel to the prepared known concentration of complex solution containing calcium chloride or calcium salts used as a complexing agents. Keep the gel containing somatic embryos in complex solution for 20 minutes? After forming the gel capsules wash it in water for 5 minutes (Saiprasad, 2001; Lambardi *et al.*, 2006) [7, 5].

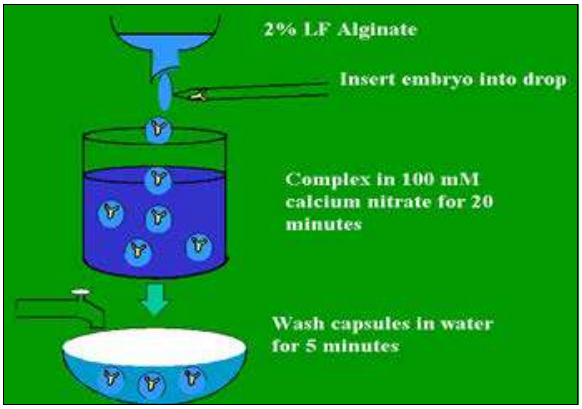

Fig 1: Dropping method of encapsulation

Molding method

This methods follows mixing of somatic embryos with temperature dependent gel (e.g. gel rite, agar).Prepare (0.5-1%) agar gel by adding agar powder into distilled water and heated it. Then insert somatic embryos in the gel and remain it for 10-15 minutes to semi solidify. Cells get coated with the gel at lowering of temperature. Then cut or excised gel containing somatic embryo either in oval shaped or square shaped of 1-2 cm.

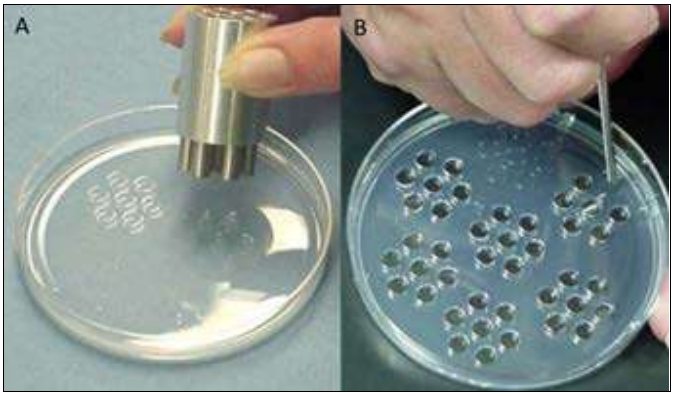

Fig 2: Moulding method of encapsulation

Procedure for artificial seed production

Selection of plant material: Select the plant material which is disease free, fresh, and can multiply or induce very fast and can give embryogenic induction.

Slice explant: Excise roots, stem, leaves, bud, embryo sac, nucellus etc as an explants.

Sterilization of explant: Different chemicals used for surface sterilization of explants are as given in the table below:

Disinfectant	Product No.	Concentration (%)	Exposure (min)
Calcium hypochlorite	211389	9-10	5-30
Sodium hypochlorite*	425044	0.5-5	5-30
Hydrogen peroxide	H1009	3-12	5-15
Ethyl alcohol	E7148	70-95	0.1-5.0
Silver nitrate	S7276	1	5-30
Mercuric chloride	M1136	0.1-1.0	2-10
Benzalkonium Chloride	B1383	0.01-0.1	5-20

Stock Solution Prepeartion: Prepare stock of macronutirent, micronutrient, vitamins, growth hormones.

Preparation of MS media: Add all the stocks like macro, micro, vitamin, hormones, agar as solidifying agent and sucrose as a source of carbon. Maintain ph and autoclave.

Inoculation of explant: Inoculate the sterilize explants in ptc jars inside laminar air flow.

Induction of callus on nutrient medium: Induction of callus in nutrient medium in growth room.

Maintenance of medium: Maintenance of medium is done to avoid damage of cells or tissue.

Transfer of callus in suspension culture: Depending upon plant species callus should transfer either to another embryogenic induced medium like MS or should transfer to suspension culture with given hormonal combination for that species for inducing embryo developmental stages.

Embryo differentiation: Differentiation of embryo into different stages.

Excision of embryo: Embryo is excised from nutrient culture medium

Preparation of gel matrix: Prepare (1-5%) sodium alginate gel or any required gel in known concentration.

Insertion of somatic embryos: Insert excised somatic embryos in sodium alginate solution and mixed it.

Preparation of complex solution: Complex solution is prepared at the con of 30-100uM, where calcium salts or calcium chloride mostly used as complexing agents.

Dropping method of encapsulation: Take sodium alginate gel containing somatic embryos in the funnel and drip the drops of gel along with somatic embryos from the tip of funnel to the prepared known concentration of complex solution containing calcium chloride or calcium salts used as a complexing agents. Keep the gel containing somatic

embryos in complex solution for 20 minutes. After forming the gel capsules wash it in water for 5 minutes.

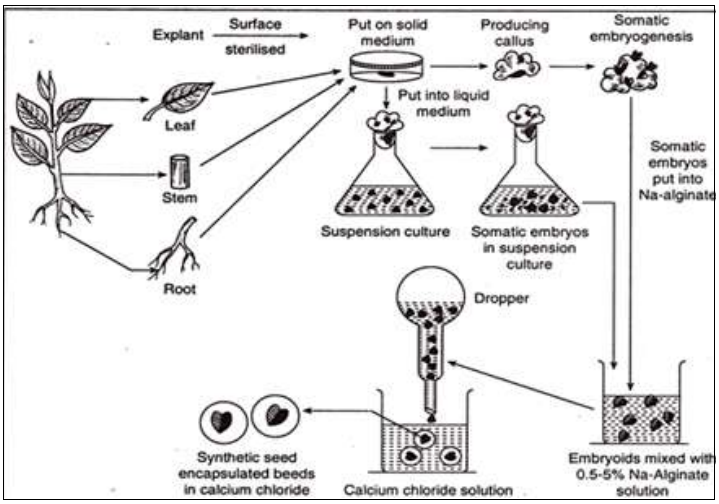

Fig 3: Stepwise procedure for artificial seed production

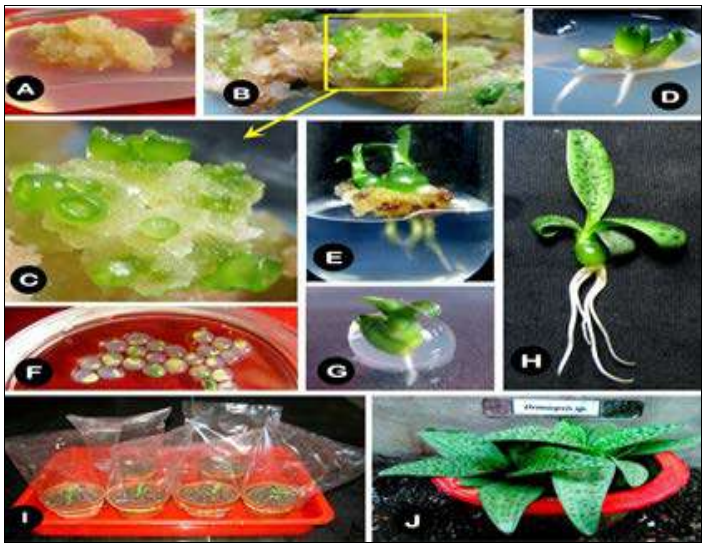

Fig: Preparation of artificial seeds from somatic embryos

Fig: Artificial seed (encapsulated somatic embryo in gel)

Encapsulat ion materials for synthetic seed

Gel concentration (% w/v)	Complexing agent	Concentration ion (uM)
Sodium alginate (0.5-5.0)	Calcium salts	30-100
Sodium alginate (2.0) with Gelatin (5.0)	Calcium chloride	30-100
Carrageenan (0.2-0.8)	Potassium chloride Ammonium chloride	500
Locust bean gum (0.4-1.0)	Potassium chloride Ammonium chloride	500
Gelrite (0.25)		

Addition of adjuvants to the matrix

Macro nutrients, micronutrients, growth regulators, vitamins, sucrose as a source of carbon or a number of useful materials such as fungicides, pesticides, antibiotics and microorganisms (eg. rhizobia) may be incorporated into the encapsulation matrix to prevent embryo from desiccation state.

Incorporation of activated charcoal into the gel matrix also improves the conversion and vigour of the encapsulated somatic embryos and it also retain the nutrients within the hydrogel capsule that results in slowly releases them to the growing embryo inside gel.

Cryopreservation of synthetic seeds

Cryopreservation is a technique for long-term storage of plant germplasm for next generations. Cryopreservation can be done at ultra-low temperature at -196 C in liquid nitrogen. The major advantage of this technique is require minimum space, rare of contamination, no need of in-vitro culture can directly grow outside the environment, stored for long period of time. The long-term conservation of embryogenic cell lines may be a valuable tool in genetic engineering. Encapsulation of seed in calcium alginate gel in *in vitro* or in *in vivo* generated explants are proved to be liable with propagation and cryostorage (Verleysen *et al.* 2005) [9].

There are several combinations of the cryopreservation techniques. The combinations of these techniques are now directly applicable for many plant species. These techniques are as follows

Slow cooling: slow cooling is achieved through programmable controlled rate freezer. Cooling temperature, initial temperature, final temperature, time limit can be set or adjusted to this freezer for slow cooling. Temperature and rate of cooling also be monitored and can be recorded on a computer.

Vitrification: It is the process of conversion of liquid phase into a non-crystalline amorphous solid preventing to form ice crystals. It is the very effective method of cryopreservation of synthetic seeds or plant materials to avoid the impairing effects of intracellular freezing.

It is commonly used for embryogenic callus lines, meristems, somatic embryos and etc. In Liquid Nitrogen. In vitrification methods, embryogenic callus/somatic embryos can be sufficiently dehydrated with PVS2 (PVS2 including 30% glycerol (w/v) + 15% ethylene glycol (w/v) + 15% dimethyl sulfoxide (DMSO; w/v) in MS (Murashige and Skoog, 1962) basal medium (plant growth regulators free) containing 0.4 M sucrose (ph 5.8).

Encapsulation: Encapsulation of embryo is achieved through hydrogels like sodium alginate, calcium alginate when get complex within complex solution which includes calcium chloride salts. These synthetic beads are usually 4 or 5mm in diameter and include one shoot tip, somatic embryos (such as globular, heart, torpedo and cotyledon stages or microcorm) or embriogenic callus clusters (Fabre and Dereuddre, 1990) [2].

Dessication (Laminal flow cabinet or silica gel): This method is easy and simple to handle. There are two desiccation techniques: dehydration under the air current of a laminar flow cabinet or dehydration in sealed containers with silica gel. Desiccation under the laminar flow can produce variable desiccation rates depending on the airflow rate, air temperature, and relative humidity.

V-Cryo-plate and D-Cryo-plate procedures: It is a new cryopreservation technique which is based on vitrification and air dehydration process of explants placed on aluminum cryo plates which are named as V-cryo-plate and D-cyo-plate technique.

D cryo-plate method: Shoot tip or buds are placed and attached to cryo plates inside the laminar air flow or over silica gel for dehydration after loading treatment with glycerol and sucrose solution for inducing tolerance to dehydration.

Applications

- Propagation of hybrid plants is very easy through artificial seeds.
- Genetically modified crops and endangered species of plants can be propagated through artificial seed technology.
- Germplasm of elite lines and endangered species can be preserved with artificial seed technology.
- Cereals crops, fruits, vegetables and medicinal plants can be studied anywhere in the world using Artificial (synthetic) seeds.
- Genetic uniformity of crops and varieties of crop can be easily maintained by using is Artificial seed technology.
- Artificial seed provides disease free conditions to plant material or explants which is present inside of capsule.
- During the production of artificial (synthetic) seed encapsulation herbicides can be added to the formulation, this herbicide will provide extra protection to the explants against pests and diseases.
- In cross pollinated crops like maize where the production of hybrids is wide spread practice. Artificial seed technology helps in production of hybrids without creation of parental lines that are costly and time consuming.
- Synthetic seed crops are easy to maintain because of uniform genetic constituent.
- Artificial seed technology improves the food production and also produces environment friendly plantation.

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