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Artificial seed technology: A brief review

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Abstract
Artificial seed/synthetic seed is defined as the encapsulation of micropropagules (somatic embryo, shoot buds /shoot tips, cell aggregates, calli, nodal segments, embryonic masses, protocorms) of a plant with specified coating material, in which the coating material acts as protection and nutrient provider to the encapsulated plant tissue, mainly preferred out covering material was calcium alginate gel which enhances the capsule formation and sufficient firmness to overcome mechanical injuries to the propagules. Nutrients, growth regulators, antibodies are incorporated into the covering material to comfort normal growth of plant propagules. It ensures the mass production of elite plant varieties. This are used for large scale multiplication of commercially valuable plants which are difficult to propagate with conventional breeding methods. It helps in the preservation of economically important plant species through cold storage and cryo preservation. This review aims to express the artificial seed production, various applications in fruit crops and limitations.

Keywords: Synthetic seed, somatic embryos, calcium alginate, encapsulation

Introduction
The most common method of propagation is seed, but in some crops propagation through seed is unsuccessful due to some reasons like seed heterozygocity, minute size, seed mortality, absence of endosperms and absolute necessity of fungal infection [1]. Another some reasons are the vegetative propagation by conventional methods are time taking long process, some are expensive and not able to produce large scale of production. The demand of artificial seed started after the discovery of the somatic embryo production in various plant species *in vitro*. The syn seeds are firstly discovered by murashinge [2], he says artificial seed is an encapsuled single somatic embryo. An artificial a somaic embryo that is engineered for the practical use in commercial plant production by Gray *et al*., [3]. Various plant materials are used for artificial seed production including somatic embryos, shoot tips, axillary buds, nodal segments, protocorms [4-9]. A typical seed consists of embryo, along with endosperm which serves as a nutrient supply for embryo growth and seed coat which contains one or more protective layers that ensures the seed. Where as in the synthetic seed the artificial coating material (sodium alginate, agar, gelrite, sodium pectate) which acts as seed coat by encapsuling the somatic embryo. The somatic embryo can be encapsulated and used like a natural seed suggested by murashinge [2].

Artificial Seed Concept
The artificial seed consists of endosperm like as in the conventional seed. It contains both explant material and the capsule (gelagent and additional materials such as nutrients, growth regulators, anti pathogeenic, bio controllers) which acts as seed coat [16].

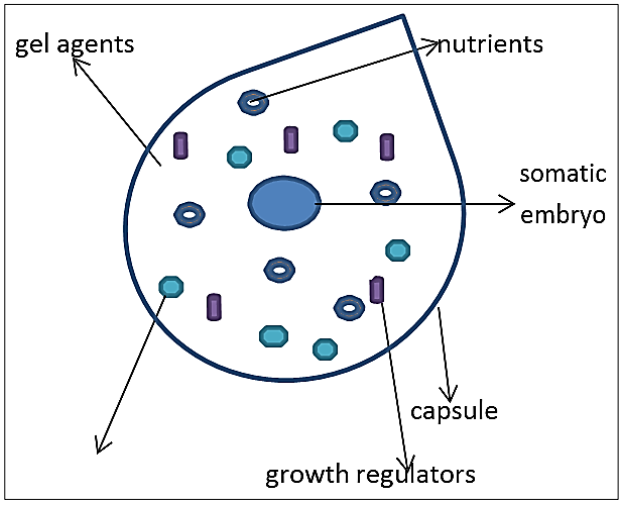

Fig 1: Antibiotic and antifungal agents

Artificial seed types

There are mainly two types of artificial seeds

1. Desiccated seed
2. Hydrated seed

Desiccated seed

This includes the encapsulation of multiple somatic embryos in the polyoxyethylene glycol followed by desiccation. Desiccation can be done by means of leaving the artificial seed on the bench for drying overnight or by slowly reducing the relative humidity in controlled period of time [4]. The desiccation is done in only in the somatic embryos which are desiccant tolerant [11]. Normally the desiccation means the storage of somatic embryo by removing the moisture. Kitto and Janick (1982) [13, 39] they encapsulated the carrot somatic embryo followed desiccation for the first time. The vigor of the seedlings from derived somatic embryos is more than the embryo which are not derived, but considerably lower than from the true seed [13]. The desiccated seeds up to 10-15% can be stored at the room temperature up to one year without cell viability and germination potential.

Hydrated seeds

Hydrated seeds contains somatic embryos or other plant tissues which are enclosed by hydrogel. So many substances like potassium alginate, agar, sodium pectate are been tried but calcium alginate is more effective in the covering of hydrated synthetic seed [14]. For production of hydrated seeds, the somatic embryos are mixed with the sodium alginate gel (0.5-5.0% W/V) which is followed by dropping into the calcium chloride solution (30-100 um) using pipette. Round and firm beads of calcium alginate contains somatic embryos are formed as the ion exchange occurs resulting in the replacement of sodium ions with the calcium ions [15]. The hardness and rigidity of the capsule depends upon the exchange of sodium and calcium ions.

Syn seed production procedure

Initiation of somatic embryogenesis

To implement synthetic seed technology in micro propagation, requires vigorous somatic embryos, initiation of somatic embryos is occurs by means of initiative treatments are given by phytohormones. The most commonly used growth regulator is auxin which initiates somatic embryogenesis. The most commonly used auxin is 2, 4-Dichlorophenoxy acetic acid (2, 4-D). Other auxins may require certain species (Ammirato, 1983) [17]. In the carrot the single cell forms the whole cluster of cells in the presence of auxin. The single cells are predetermined for embryogenesis. At this stage the cluster gets ability to develop into embryo in the absence of axuin, causes the development of stage I cell clusters [18, 19]. After the process of embryos it goes on the development stages like globular, heart shaped and torpedo shaped stages. Somatic embryogenesis is initiated mainly by auxin [20].

Development of somatic embryos

As the somatic embryogenesis is studied in many plants, the ability of somatic embryo is very less than the seed embryo because the somatic embryo is not fully developed where as in seed embryo which under goes the stage of embryo maturation. To overcome this the somatic embryos are transferred to the media containing low concentration or devoid of 2-4D is essential [17, 21, 22]. The final stage of maturation is done by transfering them to ABA (absesic acid). ABA prevents the development of embryo by supressing the secondary embryogenesis and is reported to promote embryo maturation in several species [17, 13] were able to conter desiccation tolerance alfalfa somatic embryo by treating with ABA. Bucheim *et al*., (1989) observed the initiation of soyabean somatic embryos increases from 50 to 96% when matured in presence of 10% sucrose.

Covering or encapsulation of somatic embryo

Somatic embryo requires some mechanical strength for planting, so it is more prefer to be encapsuled. The requirements for encapsulation is mentioned below:

Materials used for explant

Mostly somatic embryos are used because they has the radicle and plumule which are able to develop into root and shoot in single step [12, 4]. After that some vegetative propagules used i.e, shoot tip in M. indica [24], axillary buds in Camellia sinensis [25], bulblets in *A. sativum* [26].

Encapsulating agents

There are mainly 8 componds tested for synthetic seed coats a water soluble regin, polyox was the most suitable agent for encapsulation [12].

Reden baugh *et al*., (1987) [15, 29] proposed sodium alginate was more suitable than other compounds in some species like celery, cauliflower and carrot. Sodium alginate is mostly accepted hydro gel covering agent due to its low toxicity, low cost and bio compatible characters [1]. The several gelling agents used in the previous study are polyox, polysco 2133, agar alginate, carboxy methylcellulose, sodium pectate [1, 27].

Procedure of encapsulation

The commonly known method for encapsulation is developed by Redden baugh *et al*., (1987) [15, 29]. In this method sodium alginate of different concentration (2 to 5%) are taken and mixed in the calcium free Ms Medium and the explant is added to that solution. Then the explants are sucked out with the pipette along with the solution and dipped into calcium chloride solution were the reaction takes place of sodium ions replaces with the calcium ions which causes calcium alginate beads. The capsule size depends on the size of the pipette nozzle [28]. Molle *et al*., (1993) [29] suggested to use dual nozzle pipette where embryo passes through the inner nozzle and comes out through the outer nozzle so that the embryo was in the center of the nozzle to get better protection.

Syn seed in fruit crops

In most of the fruit crops which have commercial value, seed production have been not successful due to heterozygocity, minute size, low germination rate and some are desiccate sensitive and recalcitrant seeds which cannot be stored for long time [8]. Some of the syn seeds introduced in fruit crops are mentioned below:

Table 1: Syn seed in fruit crops

Name of the species	Type of explant used	Reference
Mangifera indica L.	Somatic embroys	Ara *et al*., 1999 [5]
Musa balbisiana kluai hin (BBB group)	Microshoots	Kanchanapoom and promsorn
Musa paradisiac L.	Shoot tips	Ganapathi *et al*., 2002 [32, 36] Hassanein *et al*., 2005 [33, 34] Hassanein *et al*., 2011 [33, 34] Matsumoto *et al*., 1995 [19, 35] Rao *et al*., 1993 [25, 32, 36]
Carica papaya L.	Somatic embryos	Castillo *et al*., 1998 [37]
Citrus nobilis slour x cdeliciosa tenora	Somatic embryos	Singh *et al*., 2007 [38] Kitto and Janick 1980 [13, 39]
Citrus reticulata blanco	Somatic embryos	Antonietta *et al*., 1999 [40]
Malus pumila Mill.M26 apple root stock	Apical buds Nodal micro cuttings Buds	Micheli *et al*., 2002 [41] Gardi *et al*., 1999 [42] Standari and Piccioni, 1997 [41, 42, 43] Gardi *et al*., 1998 [42]
Pine apple Comosus L.	Micro shoots	Gango Padhyay *et al*., 2005 [44]
		Akhtar *et al*., 1997 [45] Rai and Jaiswal, 2008 [9, 46] Rai *et al*., 2008 [9, 46]
Punica granatum L.	Nodal Segments	Naik and Chand, 2006 [47]
Vitis vinifera L.	Somatic embryos	Nirala *et al*., 2010 [48, 49] Das *et al*., 2006 [48, 49]

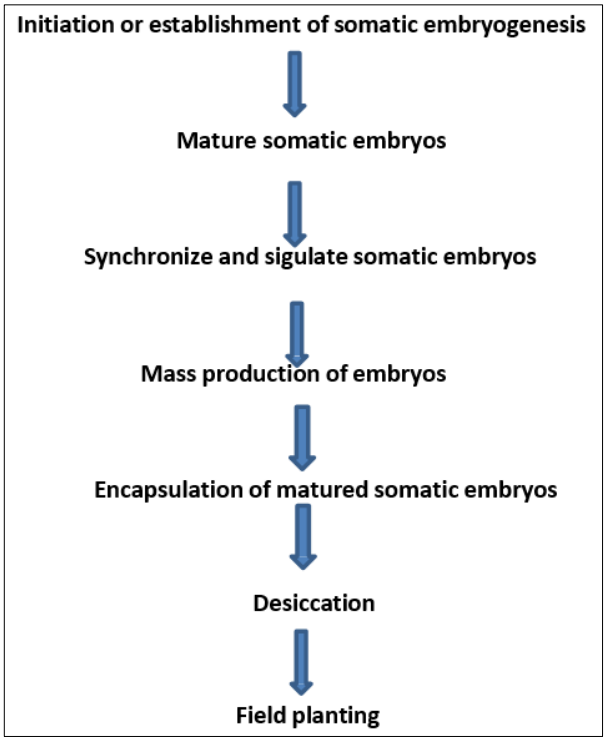

Fig 2: Steps involved in somatic embryogenesis

Limitations

As there are some limitations for the syn seed, they can be used in large scale production and conservation of rare plants, but for production of good quality micro propagules are required to produce syn seeds. But there is a limitation in the germination of viable micropropagules. The mostly used somatic embryo should attain its maturity to germinate. The immaturity leads to low germination rate [4]. The important aspects of synthetic seed technology is to conversion of synthetic seed to plantlets, due to many reasons the conversation is not possible and not able to implement the technology commercially. The coating material used for encapsulation is also a factor for the germination of plants, it also reduces the rate of conversion, the material should not get damaged, gives protection and nutrients to the developing embryo [14]. The storage place also affects the growth rate, the syn seed stored in the low temperatures reduces the viability of seed and conversion into new plant [29].

Conclusion

The Artificial Seed Technique which is widely used in conservation and delivery of tissue cultured plants. It is an alternative to the slow and costly conventional method which produces more number of plants rapidly, it also gives good potential in micro propagation in the conservation of important plant species, the lack of productivity in the micropropagules which converts into plant rate Is low so use of the syn seed in the commercial field is not possible, future studies on the syn seed will solve all the problems and used in large scale commercially.

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