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Review

Synthetic seeds: A review in agriculture and forestry

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Production of synthetic seeds has unraveled new vistas in *in vitro* plant propagation technology, because it offers many useful advantages on a commercial scale for the propagation of a variety of crop plants. These tools provide methods for production of synthetic seeds for conversion into plantlets under *in vitro* and *in vivo* conditions. This technology is useful for multiplying and conserving the elite agricultural and endangered medicinal plant species, which are difficult to regenerate through conventional methods and natural seeds. The synthetic seed technology was developed in different economically important plant species such as vegetable crops, forage legumes, industrially important crops, cereals, spices, plantation crops, fruit crops, ornamental plants, orchids, medicinal plants and wood yielding forest trees etc. All these aspects are presented in this review.

Key words: Synthetic seeds, *in vitro*, *in vivo* plant propagation.

INTRODUCTION

Nowadays, artificial seed technology is one of the most important tools to breeders and scientists of plant tissue culture. It has offered powerful advantages for large scale mass propagation of elite plant species. In general, synthetic seeds are defined as artificially encapsulated somatic embryos, shoot tips, axillary buds or any other meristematic tissue, used for sowing as a seeds and possess the ability to convert into whole plant under *in vitro* and *in vivo* conditions and keep its potential also after storage (Capuano et al., 1998). The somatic embryo can be encapsulated, handled and used like a natural seed was first suggested by Murashige (1977) and efforts to engineer them into synthetic seed have been ongoing ever since Kitto and Janick (1982),Gray (1987).

Bapat et al. (1987) 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. An implementation of artificial seed technology to somatic embryogenesis or the regeneration of embryos is based on the vegetative tissues as an efficient technique that allows for mass propagation in a large scale production of selected genotype (Ara et al., 2000). The aim and scope for switching towards artificial seed technology was for the fact that the cost-effective mass propagation of elite plant genotypes will be promoted. There would also be a channel for new transgenic plants produced through biotechnological techniques to be transferred directly to the greenhouse or field.

The artificial seed technology has been applied to a number of plant species belonging to angiosperms. Present review aimed to give a brief description of methodology involved in synseed preparation, types of synthetic seeds, species in which this technique has been developed successfully.

SYNTHETIC SEEDS PREPARATION

Induction of somatic embryogenesis

One prerequisite for the application of synthetic seed technology in micropropagation is the production of high quality vigorous somatic embryos. Induction of somatic embryogenesis requires a change in the fate of a

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vegetative (somatic) cell. In most cases, an inductive treatment is required to initiate cell division and establish a new polarity in the somatic cell. The inductive treatment is most commonly given by phytohormones. Among the plant growth regulators, auxins are known to be essential for the induction of somatic embryogenesis. In some plant species, 2,4-dichlorophenoxyacetic acid (2,4-D) is the most commonly used auxin. Other auxins may be required for certain species (Ammirato, 1983). Embryogenic cells are unique: superficially they resemble meristematic cells, though they generally are smaller, more isodiametric in shape, have larger, more densely staining nuclei and nucleoli, and have a denser cytoplasm (Williams and Maheswaran, 1986; Carman, 1990). In the carrot model described by Komamine et al. (1992), competent single cells formed embryogenic cell clusters (State 1) in the presence of auxin. These single cells are considered as predetermined for embryogenesis. During this phase, the cell clusters gained the ability to develop into embryos when auxin was removed from the medium, leading to the development of state 1 cell clusters (Nomura and Komamine, 1985; Komamine et al., 1992).

Once the induction of an embryogenic state is complete, the mechanisms of pattern formation that lead to the zygotic embryo are common passing through globular, heart-shaped and torpedo-shaped stages. Somatic embryo induction is usually promoted by auxins (Williams and Maheswaran, 1986). In some plant species, a combination of 2,4-D or α- napthalene acetic acid (NAA) with cytokinin was reported to be essential for the induction of somatic embryogenesis (Kao and Michayluk, 1981; Gingeas and Lineberger, 1989; Schuller et al., 1989). The process can be enhanced with the application of osmotic stress, manipulation of medium nutrients, reducing humidity etc.

Suspension culture

The suspension culture is mainly used for large scale production of viable materials (somatic embryos) to produce synthetic seeds. In suspension, proembryogenic cell clusters form and can be separated from the single cells and the larger clumps of callus by sequentially sieving through nylon membranes of 500 and 224 mm pore size. Generally the differentiation of somatic embryos in solid or liquid medium is highly asynchronous. Since synchronous embryo maturation is extremely important with regard to the artificial seed technology, several approaches have been developed to achieve it. This is achieved through selecting cells (Fujimura and Komamine, 1979) or pre-embryonic cell clusters of certain size (Altman et al., 1990) and manipulation of light and temperature (McKersie and Bowley, 1993), temporary starvation (Lee et al., 2001). Use of growth regulators to physiologically synchronize development has proved most effective (Dobrev et al., 2002).

Somatic embryo development

Even though somatic embryogenesis has been reported in several crop species, the quality of somatic embryos with regard to their conversion into plantlets has been very poor. This is because somatic embryos are normally incomplete in their development. Unlike seed embryos the somatic embryos actually do not go through the final phase of embryogenesis called ‘embryo maturation’. For the maturation of somatic embryos however, transfer to media containing a low concentration or devoid of 2,4-D was essential (Ammirato, 1983; Cheema 1989; Van der Valk et al., 1989). The final stage of maturation is achieved by transferring the embryos to a medium containing abscisic acid (ABA). ABA prevents germination and promotes normal development of embryos by suppression of secondary embryogenesis and is reported to promote embryo maturation in several species (Ammirato, 1983). Senaratna et al. (1989, 1990) were able to confer desiccation tolerance of alfalfa somatic embryos by treating them with ABA. Buchheim et al. (1989) observed that the conversion of soybean somatic embryos was increased from 50 to 96% when matured in the presence of 10% sucrose. Other physical treatments including cold, heat, osmotic or nutrient stress can elicit a similar response, presumably because they stimulate the endogenous synthesis of ABA (McKersie et al., 1990).

Encapsulation

Somatic embryogenesis is the only clonal propagation system economically viable. However somatic embryos would require mechanical strength for planting. It would be desirable to convert them into encapsulated units (synthetic seeds). Basic requirements for the encapsulation to form synthetic seeds are mentioned below.

Explants used for encapsulation

Ever since synthetic seed technique was developed, the somatic embryos were largely used because they possess the radical and plumule that are able to develop into root and shoot in one step (Kitto and Janick, 1982, 1985 a, b; Kim and Janick, 1987, 1989, 1990; Janick et al., 1989; Redenbaugh et al., 1984; Redenbaugh et al., 1991b; Gray et al., 1991; Redenbaugh, 1993; McKersie and Bowley, 1993). Later on vegetative propagules e.g. shoot tips in *M. indica* (Bapat et al., 1987), axillary buds in *Camellia sinensis* (Mondel et al., 2002), calli in *Allium sativum* (Kim and Park, 2002), bulblets in *A. sativum* (Bekheet, 2006), cell aggregates derived from Horseradish hairy roots (Repunte et al., 1995) and protocorm

like bodies in *Geodorum densiflorum* (Datta et al., 1999) were also used. In addition to the other *in vitro* derived meristematic tissues like microtubers, rhizomes and corms can also been used (Bapat and Minal, 2005).

Encapsulating agents

Eight chemical compounds were tested for the production of synthetic seed coats, 'Polyox', water soluble resin was the most suitable agent for the encapsulation of somatic embryos (Kitto and Janick 1982, 1985c). However, Redenbaugh et al. (1984, 1986 and 1987) proposed that sodium alginate was the most suitable for the encapsulation of somatic embryos in few species such as alfalfa, celery, cauliflower and carrot. Sodium alginate was the most accepted hydro-gel and frequently used as a matrix for synthetic seeds, because of its low toxicity, low cost, quick gellation and bio compatibility characteristics (Saiprasad, 2001). In the previous studies several gelling agents, such as polyox, polyco 2133, agar, agarose, alginate, carboxiy methylcellulose, carrageenan, guar gum, gelrite, tragacanth gum, sodium pectate ethylocellulose and nitrocellulose, polyacrylamide were tested for synthetic seed production (Ara et al., 2000; Saiprasad, 2001; Lambardi et al., 2006).

Synthetic endosperm

It is believed that the encapsulated synthetic seeds should contain nutrients and plant growth regulators to serve as synthetic endosperm to the encapsulated propagules which results in increase in the efficiency of viability and germination of synthetic seeds. The quality of artificial seeds depends on the temporal, qualitative, quantitative supply of growth regulators and nutrients along with an optimal physical environment (Senaratna, 1992). Murashige and Skoog medium (MS) without hormones and MS + 6-benzyladenine (BA, 4.4 µM) were used as artificial endosperm in *Morus* species (Pattnaik et al., 1995; Pattnaik and Chand, 2000). Refouvelet et al. (1998) used ½ MS + BA (5 mg/l) + NAA (0.01 mg/l) for encapsulation of axillary buds of *Syringa vulgaris*. Mariani (1992) reported that gibberellic acid (GA_3) and sucrose showed negative effect on synthetic seed germination in eggplant. Many investigators reported that the addition of fungicides (Antonietta et al., 2007b), pesticides, fertilizers, microorganisms (Rhizobia), mycorrhiza fungus (Tan et al., 1998), marine cyanobacterial extracts (Wake et al., 1992) bactericides and activated charcoal (Ganapathi et al., 1992) to the encapsulation solution will protect the encapsulated propagules from micro-organisms, to reduce the release of toxic compounds and to enhance the germination capacity of seed.

Encapsulation procedure

The most appropriate method for the preparation of synthetic seeds was the hydro-gel encapsulation method developed by Redenbaugh et al. (1987). In this method, sodium alginate of different concentrations (2 to 5%) was prepared by mixing with calcium free liquid MS medium and then the explants were mixed with the solution. Explants were sucked with a pipette along with the sodium alginate solution and dropped into the calcium chloride solution, where the ion exchange reaction occurs and sodium ions were replaced by calcium ions forming calcium alginate beads. The whole process must be done under aseptic conditions. The size of the capsule depends on the inner diameter of the pipette nozzle. The shape and texture of the beads depends on the concentration of the sodium alginate, calcium chloride solutions and duration of complexion. Molle et al. (1993) suggested the use of a dual nozzle pipette in this embryos flow through the inner pipette and the alginate solution passes through the outer pipette. As a result, the embryos are positioned in the center of the capsule for better protection.

Desiccation

Normally desiccation means to preserve by removing the moisture. Kitto and Janick (1982) for the first time encapsulated carrot somatic embryos followed by their desiccation. Desiccation tolerance is a characteristic of somatic embryos that must be induced and therefore requires a pretreatment with ABA or stress to elict the desired response (Helal, 2011). Fujii et al. (1993) achieved the high conversion frequencies of the somatic embryos of celery by adding ABA and mannitol to the maturation medium. The survival of coated celery somatic embryos could be improved from 35 to 86% as reported by Kim and Janick (1987) and Janick et al. (1989). The vigor of the seedlings from dried somatic embryos was greater than those from embryos which had not been dried, but remained substantially lower than those from true seeds (Senaratna et al. 1990)

Germination and field planting

The synthetic seeds have the possibility of being an alternative planting material meant for forestry sector in the future, especially for the highly demanded species (Asmah et al*.,* 2011). Artificial seeds would allow direct planting of plant propagules into the greenhouse or field, thus bypassing many of the intermediate steps. Fujii et al. (1989) found that maturation of alfalfa somatic embryos with ABA showed high soil conversion of 48 to 64%. Addition of fungicide to the alginate beads prevents the contamination and increased the survival of mulberry buds when sown in soil (Bapat and Rao, 1990). Encapsulated somatic embryos (artificial seeds) and naked (uncoated) somatic embryos of alfalfa (*Medicago sativa*) were planted directly into the field to demonstrate

the feasibility of using artificial seeds for direct sowing (Fujii et al., 1992) and reported successful field planting of alfalfa artificial seeds derived from embryoids encapsulated in calcium alginate with 23% plant conversion. Preliminary experiments on calcium-sodium alginate encapsulation of somatic embryos from anther culture were performed, in order to evaluate the effect of this technique on the recovery of plantlets, and the applicability of the synthetic seed technology to *Citrus reticulata* (Antonietta et al., 1999). However sowing on soil mix medium did not result in satisfactory conversion. The behavior of sugarcane plants cv. CP-5243 derived from artificial seed compared with traditional and isolated bud methods was studied by Nieves et al. (2003). They reported that plants from artificial seed were taller and had a smaller diameter at eight months, but these differences disappeared at 12 months. With respect to sugar analysis and yield, no differences in all parameters evaluated were found between artificial seed-derived plants and plants derived from the two other methods. Successful field planting and conversion of artificial seeds were reported by several authors (Datta et al., 1999; Mandal et al., 2000; Anand and Bansal, 2002; Martin, 2003; Ikhiaq et al., 2010). The effective germination rate in *Acacia* hybrid (73.3 to 100%) and in *Pseudostellaria heterophylla* (80%) were reported by Asmah et al. (2011) and Ma et al. (2011).

Types of synthetic seeds

According to the available literature, two types of synthetic seeds were developed, that is, desiccated and hydrated synthetic seeds (Bhojwani and Razdan, 2006). The desiccated synthetic seeds were first introduced from somatic embryos either naked or encapsulated in polyox followed by their desiccation (Kitto and Janick, 1982, 1985a, b).

Desiccation was achieved either slowly over a period of one or two weeks sequentially using chambers of decreasing relative humidity or rapidly by leaving the Petri dishes overnight on the bench of laminar airflow chamber (Ara et al., 2000). The hydrated synthetic seed technology was first produced by encapsulating hydrated somatic embryos of *M. sativa* (Redenbaugh et al., 1984). These hydrated synthetic seeds are used to produce plant species that their somatic embryos are recalcitrant and sensitive to desiccation. Hydrated artificial seeds are normally prepared by encapsulating the somatic embryos or other propagules in a hydro gel capsules. Several methodshave been examined to produce hydrated artificial seeds of which calcium alginate encapsulation has been mostly used (Redenbaugh et al., 1993).

Synthetic seeds

Synthetic seeds in plant propagation were successfully studied in number of the plant species (Table 1). Plant propagation using artificial or synthetic seeds derived from somatic embryos or other vegetative propagules opens up new vistas in agriculture and forestry. Here, according to the convenience and importance of the plant species, artificial seed technology was developed and categorized into different groups.

Vegetable crops

The production of synthetic seeds was by the encapsulation of multiple carrot somatic embryos (Kitto and Janick, 1982). In *Daucus carota*, production of desiccated synthetic seeds, hydrated synthetic seeds by using somatic embryos were reported (Kitto and Janick., 1982, 1985 a, b; Janick et al., 1989; Liu et al., 1992; Janick et al., 1993; Timbert et al., 1995; Timbert et al., 1996; Sakamoto et al., 1992 and Latif et al., 2007). 100% germination of encapsulated axillary buds by adding 0.5 mg/l NAA and 1.0% activated charcoal and advanced synthetic seed production systems by using somatic embryos in *Ipomoea batatas* were reported (Jeon et al., 1986; Cantliffe, 1993, Onishi et al., 1992, 1994). Encapsulation of celery and cauliflower somatic embryos and their conversion into plantlets were studied (Redenbaugh et al., 1986; Onishi et al., 1992). Propagation of S*olanum melongena* through encapsulation of various explants (somatic embryos, nodal segments with buds) and effect of carbon source on encapsulated propagules were described (Rao and Singh, 1991; Huda and Bari, 2007; Huda et al., 2007; Huda et al., 2009) and reported that sucrose showed better performance when nodal segment with axillary bud was used as explant, but when somatic embryos were used, sucrose + sorbitol (1:1) was found to be efficient. Encapsulation of different explants (somatic embryos, plantlets, cell aggregates from hairy roots), conservation of root regeneration potential of cell aggregates in coated capsules even after stored at 25°C up to 60 days and plant regeneration from them were observed respectively in *Armoracia rusticana* (Shigeta and Sato, 1994; Nakashimada et al., 1995; Repunte et al., 1995).

Encapsulation of different explants (somatic embryos, nodal segments, shoot tips and cell suspension cultures) and estimation of yield and canopy of field cultivated plants derived from synthetic seeds of *Solanum tuberosum* (Sarkar and Naik, 1998a; Fiegert et al., 2000; Nyende et al., 2003; Schafer-menuhr et al., 2003; Nyende et al., 2005 and Sarkar and Naik, 1998b) were investigated. Phonkajornyod et al. (2004) reported the dry synthetic seed production and desiccation tolerance induction in somatic embryos of *Capsicum annuum*. Encapsulation of nodal segments and shoot tips of *Manihot esculenta* (Cassava) germplasm was reported (Danso and Ford-iioyd 2003; Cid et al., 2009). Malek (2009) studied the propagation of *Trichosanthes*

Table 1. List of plants and type of explants used for synthetic seeds.

Name of the species	Type of explant	Reference
Acacia mangia Willd x *A. auriculaformis* Cunn. ex Benth	Shoots and axillary buds	Asmah et al., 2011
Acca sellowiana (O.Berg) Burret	Pregerminated somatic embryos	Inocente et al., 2007
Actinidia deliciosa Liang & Ferguson	Shoot buds	Piccioni and Standardi, 1995
	Nodal	Gardi et al., 1999
	In vitro derived bubs	Adriani et al., 2000
	Mirocuttings	Romay et al., 2002
Adhatoda vasica Nees	Shoot buds	Anand and Bansal, 2002
Agave vera-cruz Mill.	Shoot tips and somatic embryos	Tejavathi et al., 2006
Allium satium L.	Calli	Kim and Park, 2002
	Bulblets	Bekheet, 2006
Ananas comosus L. Merr.	Micro shoots	Soneji et al., 2002
Anthurium andreanum Lind.	Embryogenic calli	Nhut et al., 2004
	Organogenic calli	Onishi et al., 1992
Apium graveolens Dulce	Somatic embryos	Redenbaugh et al., 1986
Arachis hypogaea L.	Somatic embryos	Padmaja et al., 1995
Armoracia rusticana, Gaertn., Mey & Scherb	Hairy roots	Uozumi et al., 1992
	Somatic embryos	Shigeta and Sato, 1994
	Shoots derived from hairy roots	Nakashimada et al., 1995
	Cell aggregates of hairy roots	Repunte et al., 1995
Arnebia euchroma (Royle) Johnston	Cotyledonory stage embryos	Manjkhola et al., 2005
Asparagus cooperi Baker	Somatic embryos	Ghosh and Sen, 1994.
Asparagus officinalis L.	Somatic embryos	Mamiya and Sakamoto, 2001
Avena sativa L.	Somatic embryos	Redenbaugh et al., 1987
Bacopa monnieri L.	Nodal microcuttings	Ramesh et al., 2009
Begoniax hiemalis Forch	Somatic embryos	Awal et al., 2007
Beta vulgaris L.	Somatic embryos	Tsai and Saunders, 1999
Betula pendula Roth.	Shoot buds	Piccioni and Standardi, 1995
Betula platyphylla Sukaczev var. japonica	Axillary buds	Kinoshita and Saito, 1990
Brassica campestres L.	Apical shoot buds	Arya et al., 1998
Brassica oleracea L. var. *botrytis*	Somatic embryos	Redenbaugh et al., 1986
Camellia sinensis (L.) O.Kuntze	Nodal explants	Mondal et al., 2002
Camellia japonica L.	Somatic embryos	Janeiro et al., 1997
	Shoot tips, nodal segments	Ballester et al., 1997
Cannabis sativa L.	Axillary buds	Lata et al., 2009
	Nodal segments	Lata et al., 2011
Capsicum annuum L.	Somatic embryo	Phonkajornyod et al., 2004
Carica papaya L.	Somatic embryos	Castillo et al., 1998
Catharanthus roseus (L.) G.Don	Somatic embryos	Maqsood et al., 2012

Table 1. Contd.

Cedrela fissilis Vellozo	Shoot tips	Nunes et al., 2003
Cedrela odorata L.	Shoot tips, axillary buds	Maruyama et al., 1997a
	Shoot tips	Maruyama et al., 1997b
Celogyne odoratissima var.*angustifolia* Lindil.	Protocorms	Kamalakannan et al., 1999
Chlorophytum borivianum Sant. et Fernand.	Shoot buds	Dave et al., 2004
Cineraria maritima L.	Microshoots	Srivastava et al., 2009
Cinnamomum zeylanicum (Linn.) Cor.	Shoot buds/Somatic embryos	Sajina et al., 1997
Citrus nobilis Lour. × *C. deliciosa* Tenora	Somatic embryos	Singh et al., 2007
Citrus reticulata Blanco.	Somatic embryos	Kitto and Janick, 1980 Kitto and Janick, 1985c Germana et al., 1999 Antonietta et al., 1999 Antonietta, et al., 2007b
Cleopatra tangerine L.	Embryos	Nieves et al., 1998
Coelogyne breviscapa Lindi	Protocorm like bodies	Mohanraj et al., 2009
Coffea arabica L.	Shoot buds	Nassar, 2003
Coptis chinensis Franch	Somatic embryos	Ke et al., 1995
Coriandrum sativum L.	Somatic embryos	Chen et al., 1991; 1995
	Somatic embryos	Stephen and Jayabalan, 2000
Corymbia torelliana (F. Muell.) Hill & Johnson. 2x *C. citriodora* (Hook.) Hill and Johnson	Shoot tips and Nodes	Hung andTrueman, 2011
Crataegus oxyacantha L.	Nodal segments	Piccioni and Standardi, 1995
	Shoot buds	Piccioni and Standardi, 1995
Cremastra appendiculata (D.Don) Makino	Protocorm	Zhang et al., 2009
Cucumis sativus L.	Somatic embryos	Tabassum et al., 2010
Curcuma longa L.	Adventatious buds	Sajina et al., 1997
Cyclamen persicum Mill.	Somatic embryos	Winkelmamm et al., 2004
Cymbidium giganteum Wall ex Lindl.	Protocorm	Corrie and Tandon, 1993
	Somatic embryos	Nhut et al., 2005
Dalbergia sissoo Roxb.	Somatic embryos	Singh and Chand, 2010
	Nodal segments	Chand and Singh, 2004
Daucus carota L.	Somatic embryos	Kitto and Janick, 1985a
	Somatic embryos	Timbert et al., 1996
	Somatic embryos	Wake et al., 1992
	Somatic embryos	Latif et al., 2007
	Somatic embryos	Sakamoto et al., 1992
	Asexual embryos	Kitto and Janick, 1985b
	Somatic embryos	Liu et al., 1992
	Somatic embryos	Janick et al., 1989
	Somatic embryos	Timbert et al.,1995
	Somatic embryos	Kamada et al., 1989
	Somatic embryos	Kitto and janick, 1982
	Somatic embryos	Onishi et al., 1994

Table 1. Contd.

Dendranthema x grandiflora (RAMAT)	Nodal segments	Pinker and Abdel-rahman, 2005
Dendrobium huoshanense Tang ex Cheng.	Protocorm like bodies	Saiprasad and Polisetty, 2003
Dendrobium densiflorum Lindl.	Protocorm like bodies	Vij et al., 2001
Dendrobium wardianum Warner	Protocorm like bodies	Sharma et al., 1992
Dendrocalamus strictus L.	Somatic embryos	Mukunthakumar and Mathur, 1992
Dianthus caryophyllus L.	Shoot tips	Halmagyi and Deliu, 2007
Dioscorea bulbifera L.	Shoot tips	Narula et al., 2007
Dioscorea spp	Nodal segments	Hasan and Takagi, 1995
Olea europaea L. Shift	Nodal explants	Gardi et al., 1999
Elaeis guineensis Jacq.	Somatic embryos	Mariani et al., 2008
Elettaria cardamom Maton	Shoot tips	Ganapathi et al., 1994
Eleusine coracana Gaertn	Somatic embryos	George and Eapen, 1995
Eucalyptus citriodora Hook	Somatic embryos	Muralidharan and Mascarenhos, 1995
Eustoma grandiflorum (Raf.) Shinners	Somatic embryos	Ruffoni et al., 1994
Flickingeria nodosa (Dalz.) Seidenf.	Protocorm like bodies	Nagananda et al., 2011
Genista monosperma Lam.	Somatic embryos	Ruffoni et al., 1994
Geodorum densiflorum (Lam) Schltr.	Protocorm like bodies	Datta, et al., 1999
Gerbera jamesonii Bolus ex Hook.f	Microshoots and somatic embryos	Taha et al., 2009a
Gladiolus cultivars	Somatic embryos	Ramakrishnappa, 1998
Goiabeira serrana	Somatic embryos	Guerra et al., 2001
Guazuma crinita Mart	Shoot tips	Maruyama et al., 1997b
	Shoot tips, axillary buds	Maruyama et al.,1997a
Gypsophila paniculata L.	Shoot tips	Rady and Hanafy, 2004
Helianthus annuus L.	Shoot tips	Katouzi et al., 2011
Hibiscus moscheutos Welw. ex Hiern.	Nodal segments	Preece and West, 2006
Hopea parviflora Bedd.	Excised embryos	Sunilkumar et al., 2000
Hordeum vulgare L.	Microspore derived embryos	Datta and Potrykus, 1989
Hyoscyamus muticus L.	Somatic embryos	Pandey and Chand, 2005
Ipomoea aquatica Forsk	Axillary buds	Shaohu et al., 1994
Ipomoea batatas L.	Somatic embryos	Chee and Cantlieffe, 1992.
	Somatic embryos	Cantliffe, 1993
	Somatic embryos from true seeds	Jeon et al., 1986
	Somatic embryos	Cantliffe et al., 1987
Ipsea malabárica (Reichb. F.) J. D. Hook.	Bulbs	Martin, 2003
Jacaranda mimosaefolia D.Don	Shoot tips or axillary buds	Maruyama et al., 1997a
	Shoot tips	Maruyama et al., 1997b
Kiwifruit CV Hayward, Tomuri	Nodal	Gardi et al., 1999
Laeliocattleya okarchee Addison (Cattleya)	Protocorm like bodies	Saiprasad and Polisetty, 2003
Lilium longoflorum Thunb.	Bulblets	Standardi et al., 1995
Eustoma grandiflorum (Raf.) Shinn.	Somatic embryos	Ruffoni et al., 1993
Lycopersicon esculentum Mill.	Seeds	Garrett et al., 1991
Malus pumila Mill. M 26 apple root stock	Apical buds	Micheli et al., 2002
	Nodal micro cuttings	Gardi et al., 1999

Table 1. Contd.

	Micropropagated buds	Piccioni, 1997
	Buds	Standardi and Piccioni, 1997
	Shoot tips	Sicurani et al., 2001
	Machine produced cuttings	Brischia et al., 2002
	Apical and axillary buds	Capuano et al., 1998
	Nodal explants	Gardi et al., 1999
	Micropropagated buds	Piccioni and Standardi, 1995
Mangifera indica L.	Somatic embryos	Ara et al., 1999
Manihot esculenta L.	Axillary buds	Cid et al., 2009
	Nodal segments and shoot tips	Danso and Ford-Lloyd, 2003
Medicago sativa L.	Somatic embryos	McKersie et al., 1989 Fujii, et al., 1992. Redenbaugh et al., 1986 Mckersie et al., 1990 McKersie and Brown, 1996 Redenbaugh et al., 1984, Redenbaugh and Walker 1990 McKersie and Bowley, 1993 Senaratna et al., 1990
Mentha arvensis L.	Axillary buds	Ahuja et al., 1989
Morus alba L.	Axillary buds	Pattnaik and Chand, 2000
	Axillary buds	Bapat, 1993
	Axillary buds	Machii, 1992
Morus australis Poir.	Axillary buds	Pattnaik and Chand, 2000
Morus bombycis Koidz	Axillary buds	Pattnaik and Chand, 2000
Morus cathyana Hemsl	Axillary buds	Pattnaik and Chand, 2000
Morus indica L.	Shoot buds	Bapat et al., 1987
	Axillary buds	Bapat and Rao, 1990
	Axillary buds	Kavyashree et al., 2006
	Apical buds	Kavyashree et al., 2004
Morus latifolia Poir.	Axillary buds	Pattnaik and Chand, 2000
Morus nigra L.	Axillary buds	Pattnaik and Chand, 2000
Morus alba L.	Axillary buds	Pattnaik et al., 1995
Musa balbisiana 'Kluai Hin' (BBB group)	Microshoots	Kanchanapoom and Promsorn, 2012
Musa paradisiaca L.	Shoot tips	Ganapathi et al., 2002 Ganapathi et al., 1992 Hassanein et al., 2005 Hassanein et al., 2011 Matsumoto et al., 1995 Priya and Arumugam, 2003 Rao et al., 1993

Table 1. Contd.

		Srinivas., 2002
		Suprasanna et al., 2000
Musa paradisica L. cv. *rasthali*	Somatic embryos	Ganapathi et al., 2001.
Musa paradisica L. cv. *grand naine*	Microshoots	Sandoval-yugar et al., 2009
Nerium oleander L.	Axillary buds	Ozden et al., 2008
Nothafagus alpina (Poepp. & Endl.) Oerst.	Somatic embryos	Cartes et al., 2009
Ocimum americanum L.	Axillary buds	Mandal et al., 2000
Ocimum basilicum L.	Axillary buds	Mandal et al., 2000
Ocimum gratissimum L.	Axillary buds	Mandal et al., 2000
Ocimum sanctum L.	Axillary buds	Mandal et al., 2000
Olea europaea L cv. *moraiolo*	Microcuttings	Micheli et al., 2007
Olea europaea L.	Nodal segments	Gardi et al., 1999
Olive cv. Camino, Moraiolo, *Ascolana tenera* and Dolce agogia	Nodal segments	Gardi et al., 1999
Olea europaea L.	*In vitro* derived shoots	Ikhiaq et al., 2010
Oncidium 'Gower ramsay'	Protocorm like bodies	Saiprasad and Polisetty, 2003
Oryza sativa L.	Protoplast cultures	Giri and Reddy, 1994
	Androgenic proembryos	Roy and Mandal., 2008
	Somatic embryos	Suprasanna et al., 1996
	Somatic embryos	Arunkumar et al., 2005
Oryza sativa L. cv. *basmati* 370	Somatic embryos	Suprasanna et al., 2002
Parkia speciosa Hassk.	Embryos	Ummi et al., 2011
Paulownia elongata Hu.	Direct somatic embryos	Ipekci and Gozukirmizi, 2003
Pelargonium domesticum Bailey	Somatic embryos	Marsolais et al., 1991
Pelargonium x hortorum (zonal geranium)	Somatic embryos	Marsolais et al., 1991
Pelargonium horturum Bailey.	Somatic embryos	Gill et al., 1994
Phaius tankervillae (Banks) Blume	Protocorm like bodies	Malemngaba et al., 1996
Phoenix dactylifera L.	Somatic embryos	Bekheet et al., 2002 ; 2005
Photinia fraseri Dress	Axillary buds	Ozden et al., 2008
Phyllanthus amarus L.	Somatic embryos	Singh et al., 2006a
Picea abies L.	Somatic embryos	Gupta et al., 1987
Picrorhiza kurroa Royle. ex. Benth.	Micro shoots	Mishra et al., 2011
Pineapple *Ananas comosus* L.	Micro shoots	Gangopadhyay et al., 2005
Piper hispidinervum C.DC.	Pre germinated seeds	Pereira et al., 2008
Piper nigrum L.	Somatic embryos	Fair and Gupta, 2007
	Shoot buds	Sajina et al.,1997
Pistachio vera L.	Embryoids	Onay et al., 1996
Plantago asiatica L.	Shoot tips	Makowczynska and Andrzejewska-golec, 2006
Pogonatherum paniceum (Lam.) Hack	Shoot buds	Wang et al., 2007
Pogostemon cablin Benth.	Nodal segments	Swamy et al., 2009
Populus tremuloides L . x *P. tremula* Michx. Hybrid aspen	Shoot tips	Tsvetkov et al., 2006
Pseudostellaria heterophylla (Miquel) Pax.	Micro-tubers	Ma et al., 2011
Psidium guajava L.	Somatic embryos	Akhtar et al., 1997
	Somatic embryos	Rai and Jaiswal, 2008
	Shoot tips	Rai et al., 2008a

Table 1. Contd.

	Nodes	Rai et a., 2008b
	Somatic embryos	Rai et a., 2008c
Punica granatum L.	Nodal segments	Naik and Chand, 2006
Pyrus communis L.	Shoot tips	Ahmad et al., 2007
	Shoot tips	Ray and Bhattacharya, 2008
Rauvolfia serpentina (L.) Benth. ex Kurz.	Microshoots	Faisal et al., 2012
	Callus	Reddy et al., 2005
Rauvolfia tetraphylla L.	Microcuttings	Faisal et al., 2006
	Microshoots	Alatar and Faisal, 2012.
Rhodiola kirilowii (Regel) Maxim	Axillary buds and callus	Zych et al., 2005
Rosa hybrida Kings Ransom	Somatic embryos	Jayasree and Devi, 1997
	Shoot buds	Piccioni and Standardi, 1995
Rubus idaeus L.	Nodal	Gardi et al., 1999
	Nodal	Gardi et al., 1999
	Microcuttings	Romay et al., 2003
Ruta graveolens L.	Roots	Vdovitchenko and Kuzovkina, 2011
		Naik and Chikkagouda, 1997
Saccharum officinaium L	Somatic embryos	Sandeep, 2008
		Boonpeng et al., 2003
Saintpaulia ionantha Wendl.	Somatic embryos	Taha et al., 2009b
	Micro shoots	Daud et al., 2008
Salvia officinalis L.	Shoot tips	Grzegorczyk and Wysokinska, 2011
		Bapat, 1993
Santalum album L.	Somatic embryos	Bapat and Rao, 1992
		Bapat and Rao, 1988
Scutellaria baicalensis Georgi	Roots	Vdovitchenko and Kuzovkina, 2011
Siberian ginseng	Somatic embryos	Choi and Jeong, 2002
Simmondsia chinensis L.	Shoot apical and axillary buds	Hassan, 2003
	Somatic embryos	Rao and Singh, 1991
Solanum melongena L.	Nodal segment with bud	Huda et al, 2007
	Somatic embryos	Huda and Bari, 2007
	Axillary buds	Huda et al., 2009
	Somatic embryos	Mariani, 1992
Solanum nigrum L.	Shoot tips	Verma et al., 2010
Solanum tuberosum L.	Nodal segments	Sarkar and Naik, 1998b

Table 1. contd.

	Shoot tips	Nyende et al., 2005
	Shoot tips	Patel et al., 2000
	Shoot tips	Nyende et al., 2005
	Nodal segments	Sarkar and Naik, 1998a
	Shoot tips	Nyende et al., 2003
	Shoot tips	Aggrey et al., 2003
Solanum tuberosum L. cv. *clarissa*	Cell suspension culture	Schafer-menuhr et al., 2003
Solanum tuberosum L. cv. *tomensa*	Somatic embryos	Fiegert et al., 2000
Spathoglottis plicata BL.	Protocorm like bodies	Singh, 1991
	Seeds and protocorms	Khor et al., 1998
	Seeds	Tan et al., 1998
	Protocorms	Singh, 1991
Stevia rebaudiana Bertoni	Shoot tips	Andlib et al., 2011
	Shoot tips, axillary buds	Ali et al., 2012
Syringa vulgaris L.	Axillary buds	Refouvelet et al., 1998
	Axillary buds	Ozden et al., 2008
Theobroma cacao L.	Embryos	Sudhakara et al., 2000
Trichosanthes dioica Roxb.	Shoot tips	Malek, 2009
Tylophora indica (Burm.f.) Merrill	Shoots	Faisal and Anis, 2007
	Somatic embryos	Chandrasekhar et al., 2006
	Somatic embryos	Devendra et al., 2011
Valeriana wallichii DC.	Apical and axial shoot buds	Mathur et al., 1989
Vanda coerulea Grifft.	Protocorm like bodies	Sarmah et al., 2010
Vanilla wightiana Lindl.	Shoot buds	Sajina et al., 1997
Vitex negundo L.	Nodal segments	Ahmad and Anis, 2010
Vitis vinifera L.	Somatic embryos	Nirala et al., 2010
		Das et al., 2006
Withania somnifera (L.) Dunal	Shoot tips	Singh et al., 2006b
Zea mays L. var *saccharata*	Somatic embryos	Thobunluepop et al., 2009
Zingiber officinale Rosc.	Shoot buds	Sharma et al., 1994
	Micro shoots	Sundararaj et al., 2010

dioica through encapsulated shoot tips. Ummi et al. (2011) investigated the effect of different storage intervals on encapsulated embryo and germination of *Parkia speciosa*. The encapsulated *P. speciosa* zygotic embryo (without storage) showed that germination occurred after seven days of culture on the germination medium. The synthetic seed stored at 4°C remained viable and germination is initiated on day 14 after culture.

Forage legumes

Among the forage legumes somatic embryogenesis and the development of synthetic seed, technology was

extensively studied in *Medicago sativa* (alfalfa). Encapsulation in hydrogel remains to be the most studied method of artificial seed production in alfalfa (Redenbaugh et al., 1986; Redenbaugh and Walker, 1990). Fujii et al. (1998) found improvement of plantlet growth and vigor through artificial seeds. Maturation and green house planting of alfalfa artificial seeds were studied by Fujii et al. (1987, 1989 and 1992) and observed that embryo maturation with ABA (5 µM) was essential for high soil conversion (50 to 64%). Mckersie et al. (1989) developed artificial seeds of hybrid alfalfa. Induction of desiccation tolerance in *M. sativa* somatic embryos by ABA treatment and production of synthetic seeds were studied by Senaratna et al. (1989, 1990). They found that somatic embryos treated with ABA showed about 60% survival and conversion into plantlets when placed on moist filter paper or sown directly onto sterile soil. However, efficient coating and encapsulation methods for desiccated embryos of *M. sativa* are yet to be developed (Redenbaugh et al., 1991a, b). Somatic embryos of alfalfa (*M. sativa*) desiccated to 10 to 15% could be stored at room temperature for one year without a decline in their germinability (Mc Kersie and Bowley, 1993).

Industrially important crops

Synthetic seed technology started from the mid 1980's in the industrially important crops such as mulberry, sandalwood, sugarcane etc. Mulberry is one of the most important crops which play an important role in silk industry, because its leaves serve as chief source for feeding silkworms (Yu et al., 2008). Bapat et al. (1987) and Bapat and Rao (1990) reported the successful *in vivo* growth of encapsulated shoot tip of *Morus indica* by the addition of fungicide to the alginate beads without contamination. Several reports have been published on axillary buds as encapsulation propagules in *Morus* spp. such as *Morus alba* (Machii, 1992), three years old mature mulbery trees of three indigenous and two Japanese varieties (Pattnaik et al., 1995) and in six mulberry species *M. alba*, *Morus australis*, *Morus bombycis*, *Morus cathyana*, *Morus latifolia* and *Morus nigra* (Pattnaik and Chand, 2000). Kavyashree et al. (2004, 2006) reported that the synthetic seeds were successfully produced by encapsulating non-embryogenic propagules like apical and axillary buds excised from asceptic shoots of mulberry (*M. indica* cv. S54). Sandalwood is a commercially valuable forest tree of India. It plays an important role in many industries such as pharmaceutical, cosmetics, soap and perfume industries etc. Plantlet regeneration from encapsulated somatic embryos of S*antalum album* was reported by Bapat and Rao (1988, 92) and Bapat (1993). *Saccharum officinarum* is well known as an extremely important commercially cultivated crop in India, which plays an important role in sugar and fermentation industries, but, normally these crops are more susceptible to many fungal and bacterial diseases and propagate by using culm cuttings of mature stems which are very expensive. So 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. A model system for synchronous somatic embryo production combined with the formation of synthetic seeds was studied by Boonpeng et al. (2003) and found that 60% of germination successfully on Murashige and Skoong medium. Nieves et al. (2003) 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.

Medicinal plants

Naturally most of the important medicinal plants are rare, endangered and endemic category. It is due to the low fruit and seed formation, poor germination capacity of seeds and due to the many other environmental conditions such as habitat modification, urbanization, climatic change and pollution etc. So, it is important to propagate and conserve these plant species. The production of synthetic seeds by encapsulating somatic embryos and vegetative propagules is rapidly becoming an applied technique with potential for mass propagation of medicinal plant species. Propagation of *Valeriana wallichii* using encapsulated apical and axial shoot buds with plantlet conversion under both *in vitro* (98%) and in *vivo* (64%) was reported by Mathur et al. (1989). Hasan and Takagi (1995) used nodal segments for encapsulation in *Dioscorea* spp. 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). Anand and Bansal (2002) found that maximum conversion of the encapsulated shoot buds of *Adhatoda vasica* was on Gamborg's medium containing 4.65 µM KN and 50 mg/l phloroglucinol. Direct somatic embryogenesis and synthetic seed production from *Paulownia elongata* was studied and their germination frequencies was reported as 53% before storage at 4°C and 43.2 and 32.4%, respectively after 30 and 60 days of storage by Ipekci and Gozukirmizi (2003). Dave et al. (2004) studied the *in vitro* propagation of rare Indian medicinal herb *Chlorophytum borivilianum* via encapsulated shoot buds and found that 80% of sprouting at 28±2°C after three weeks and 90% of sprouting even after seven days of storage at 4°C. Pandey and Chand (2005) reported the efficient plant regeneration (60%) from encapsulated somatic embryos of *Hyoscyamus muticus.*

Micropropagation of *Rhodiola kirilowii* using the encapsulated axillary buds and callus was reported by

Zych et al. (2005). They found that 100% plantlet conversion even after six weeks of storage at 4°C. Studies on different plant species using various explants, cotyledonory embryos in *Arnebia euchroma* (Manjkhola et al., 2005), somatic embryos in *Tylophora indica* (Chandrasekhar et al., 2006), shoot tips in *Phyllanthus amarus* (Singh et al., 2006a), microcuttings in *Rauvolfia tetraphylla* (Faisal et al., 2006), shoot tips in *Withania somnifera* (Singh et al., 2006b), shoot tips in *Plantago asiatica* (Makowczynska and Andrzejewska-golec, 2006), shoot tips in *Dioscorea bulbifera* (Narula et al., 2007) and shoot tips in *Rauvolfia serpentina* (Ray and Bhattacharya 2008) has been reported. Ramesh et al. (2009) studied the effect of fungicide (bavistin) on conversion from encapsulated nodal microcuttings of micropropagated *Bacopa monnieri* and reported that 3.0 mg/l bavistin showed no reduction in plant conversion and generated maximum number of shoots even after storage up to 45 days at 18°C. The conversion to plantlet from alginate encapsulated axillary buds of *Cannabis sativa* was studied by Lata et al. (2009) and found that maximum conversion under *in vitro* was on MS medium supplemented with thidiazuron (TDZ, 0.5 µM) and plant preservative mixture (PPM, 0.07%) whereas *in vivo* 1:1 potting mix fertilonue with coco natural growth medium, moistened with full strength MS + 3% sucrose + 0.5% PPM was observed. Cartes et al. (2009) encapsulated somatic embryos and zygotic embryos from mature seeds of rauli-beech (*Nothofagus alpina*) in different artificial endosperms in order to generate a cover and fulfill the function of nourishment, protection of the embryos facilitating their later germination. Srivastava et al. (2009) found the genetic stability of plants derived from encapsulated microshoots following six months of storage in *Cineraria maritima.*

Plant regeneration was achieved from encapsulated nodal segments in *Vitex negundo*, shoot tips in *Solanum nigrum* and microshoots in *Picrorhiza kurroa* (Ahmad and Anis, 2010; Verma et al., 2010; Mishra et al., 2011). Lata et al. (2011) prepared synthetic seeds of *C. sativa* by using the *in vitro* derived axillary buds and studied the genetic stability of synthetic seeds during *in vitro* multiplication and storage for six months at different growth conditions using inter simple sequence repeat (ISSR) DNA fingerprinting which showed homogenesity in the regrown clones and mother plant. Ma et al. (2011) produced a protocol for encapsulation and germination of microtubers of *P. heterophylla*. Andlib et al. (2011) reported that the synthetic seeds produced by the encapsulation of shoot tips are an alternative source for quick regeneration of *Stevia rebaudiana*. Direct somatic embryogenesis and synthetic seed production in *Tylophora indica* was reported by Devendra et al. (2011).

Cereals

The application of synthetic seed technology to the cereals started from the year 1989. Most of investigations were carried out to increase their yield and vigor. Artificial seeds are playing a major role in increasing the genetically transformed plant material and haploid plant production. Datta and Potrykus (1989) reported synthetic seeds derived from embryos of *Hordeum vulgare*. After this, Giri and Reddy (1994) reported alginate encapsulation of *Oryza sativa*. George and Eapen (1995) reported the encapsulation of somatic embryos in *Eleusine coracana*. Suprasanna et al. (1996) showed that the encapsulation of somatic embryos and conversion into plantlets of *Oryza sativa*. Suprasanna et al. (2002) studied the viability of encapsulated embryos derived from five year old long term culture of *Oryza sativa* cv. *basmati* 370. Arunkumar et al. (2005) repoprted the addition of protectants, bavistin and streptomycin as constituents of synthetic endosperm and found that there was no negative effect on germination and conversion. They also studied the conversion of synthetic seeds into seedlings in hybrid rice and reported that the application of self-breaking gel beads technology increased the germination (52%) and conversion (47%) of synthetic seeds. Roy and Mandal (2008) reported the development of synthetic seeds involving androgenic and pro-embryos in elite *Oryza sativa*. Model systems for synchronous somatic embryo production combined with encapsulation to form synthetic seeds were studied in *Zea mays* var. *saccharata* (Thobunluepop et al., 2009).

Spices and plantation crops

Chen et al. (1991) reported 82% germination capacity of artificial seeds and survival rate of 83% in *Coriandrum sativum*. Stephen and Jayabalan (2000) produced artificial seeds in *Coriandrum sativum* by using somatic embryos derived from hypocotyls explants. Production of disease free encapsulated shoot buds of *Zingiber officinale* and their conversion into plantlets were reported by Sharma et al. (1994). High frequency plant regeneration from *Allium sativum* encapsulated calli and bulblets were reported, respectively by Kim and Park (2002) and Bekheet (2006). *In vitro* plant regeneration from encapsulated somatic embryos of *Piper nigrum* was reported by Nair and Gupta (2007). Sundararaj et al. (2010) showed the microshoots encapsulation of *Zingiber officinale*. Maximum conversion of the encapsulated shoot tips of *Elettaria cardamomum* into plantlets was on White's medium (Ganapathi et al., 1994). Cold storage of shoot cultures and alginate encapsulation of shoot tips of *Camellia japonica,Citrus reticulata* and propagation of tea (*C. sinensis*) by shoot proliferation of alginate-encapsulated nodal explants stored at 4°C were reported by Janeiro et al. (1997), Ballester et al. (1997) and Mondal et al. (2002). Induction of somatic embryo-genesis, production of synthetic seeds and 70% of germination in *Elaeis guineensis* was studied by Mariani et al. (2008).

Fruit crops

In most of the commercial fruit crops, the seed propagation has not been successful because of heterogeneity of seeds; minute seed size and presence of reduced endosperm, low germination rate and in some crops have desiccation sensitive and recalcitrant seeds which cannot be stored for longer time (Rai et al., 2009). Recently many of the crops available are seedless varieties. Propagation of *Musa paradisica* (Ganapathi et al., 1992; Matsumoto et al., 1995; Hassanein et al., 2005.) and *Musa paradisica* cv. *grand naine* (Sandoval-Yugar et al*.,* 2009) was carried out through encapsulated shoot tips. In banana cv. *rasthali* (*Musa* spp. AAB group), plantlet regeneration was from alginate encapsulated somatic embryos (Ganapathi et al., 2001). Rooting induction and plantlet regeneration from *Malus pumila* var. M 26 apple rootstock synthetic seeds prepared by using apical and axillary micropropagated buds were reported (Standardi and Piccioni, 1997; Piccioni, 1997; Capuano et al*.,* 1998; Sicurani et al*.,* 2001; Brischia et al*.,* 2002). Attempts for saving labor by using mechanical tools in the production of adventitious shoot tips suitable for encapsulation were tried by Sicurani et al*.* (2001). Micheli et al. (2002) stated that the presence of second layer of alginate (double encapsulation) and of a thin external coating layer on the alginate (encapsulation coating) did not show any detrimental effects on viability, sprouting and regrowth of the encapsulated microcuttings in M 26 apple rootstock. Effect of encapsulation on *Citrus reticulata* somatic embryo and their plantlet conversion, prospective of the encapsulation technology in the nursery activity of *Citrus* were studied by different authors (Antonietta et al., 1999; Antonietta et al., 2007 a, b). As compared to non-encapsulated or encapsulated with a growth regulator free artificial endosperm, somatic embryos encapsulated with an artificial endosperm containing GA_3 has greater ability to plantlet conversion (Antonietta et al., 1999).

The addition of thiophosphate – methyl (fungicide) in the artificial endosperm resulted in high levels of sprouting with prominent frequencies of root development and conversion into plantlets. Under *in vitro* and *ex vitro* conditions, Antonietta et al. (2007b) and Singh et al. (2007) studied the effect of storage conditions on percentage of germination of encapsulated and non encapsulated somatic embryos of kinnow mandarin (*Citrus nobilis* x *C. deliciosa* Tenora). Encapsulation of different explants were reported which includes: somatic embryos in *Carica papaya* and *Mangifera indica* (Castillo et al., 1998; Ara et al*.,* 1999), micro shoots in *Ananas comosus* (Soneji et al*.,* 2002; Gangopadhyay et al., 2005), nodal segments in *Punica granatum* (Naik and Chand 2006), shoots tips in *Pyrus communis* (Ahmad et al., 2007), shoot tips in *Psidium guajava* (Rai et al., 2008a) and somatic embryos in Vitis vinifera (Nirala et al, 2010).

Forest trees

Mukunthakumar and Mathur (1992) observed that the additional coating of mineral oil on artificial seeds of *Dendrocalamus strictus* produced by encapsulating somatic embryos showed 56% plantlet conversion frequency at *in vivo* condition. The possibility for encapsulation of shoot tips and axillary buds in the production of artificial seeds in *Cedrela odorata*, *Guazuma crinita* and *Jacaranda mimosaefolia* were studied by Maruyama et al. (1997a, b). Hassan (2003) produced encapsulated shoot apical and axillary buds of *Simmondsia chinensis*. *In vitro* conservation of *Cederla fissilis* via encapsulation of shoot tips, cotyledonory and epicotyl nodal segments were reported by Nunes et al. (2003). Plant regeneration from alginate encapsulated nodal segments and somatic embryos of *Dalbergia sissoo*, respectively was described by Chand and Singh (2004) and Singh and Chand (2010). Inocente et al. (2007) reported that the pre-germinated somatic embryos encapsulated in a sodium alginate with BA (0.5 µM) and GA_3 (1 µM) developed radicals of *Acca sellowiana*. Hung and Trueman (2011) studied the alginate encapsulation of shoot tips and nodes for short term storage of *Corymbia torelliana* x *Corymbia citriodora*. They found that the seeds pretreated with indole 3 butyric acid (IBA) stored about six weeks effectively at 25°C (50 to 84% of germination) than that of 4°C (0 to 4% of germination). Highest frequencies of plantlet formation (46 to 80%) under *in vivo* conditions when pre converted shoot tip derived synthetic seeds were transferred on to an organic compost substrate and their 100% survival in nursery was described. Asmah et al*.* (2011) developed a protocol for encapsulation of *Acacia* hybrid *in vitro* derived shoots and axillary buds and found that the germination rate was within 73.3 to 100% in the duration of six to 20 days.

Ornamental plants and orchids

In ornamental plants and orchids, the synthetic seeds have very much commercial importance, because of their minute seed size and presence of reduced endosperm in seeds (Lambardi et al., 2006). Ruffoni et al*.* (1994) produced synthetic seeds of somatic embryos in two ornamental species (*Eustoma grandiflorum* and *Genista monosperma*). Piccioni and Standardi (1995) produced synthetic seeds of shoot tips in *Betula pendula* (birch). Standardi et al. (1995) produced synthetic seeds of bulbs in *Lilium longiflorum.* A new protocol was presented for producing synthetic seeds of axillary buds in *Syringa vulgaris* (Refouvelet et al., 1998). Nhut et al*.* (2004) studied the propagation of *Anthurium andreanum* by the encapsulation of embryogenic calli. Rady and Hanafy (2004) reported the synthetic seed for encapsulation and regrowth of *in vitro* derived *Gypsophila paniculata* shoot tips. In various ornamental plant species using different

explants such as: shoot tips, microshoots and axillary nodes of hybrid aspen (Tsvetkov et al*.,* 2006), *Saintpaulia ionantha* (Daud et al*.,* 2008), *Nerium oleander*, *Photinia fraseri* and *Syringa vulgaris* (Ozden et al*.,* 2008). Katouzi et al. (2011) encapsulated shoot tips of *Helianthus annuus* by adding salicylic acid for cold preservation.

In orchids the most widely used explants for the preparations of synthetic seeds are the seeds, protocorms and protocorms like bodies (Murthy et al., 2006). Khor et al*.* (1998)*,* developed two-coat system for encapsulation of *Spathoglottis plicata* seeds and protocorms. The seeds and protocorms could withstand the encapsulation treatment with high viability 64 and 40%, respectively. Datta et al. (1999) produced an encapsulation of protocorm like bodies in *Geodorum densiflorum* an endangered orchid. Martin (2003) produced synthetic seeds in *Ipsea malabarica* by using *in vitro* formed bulbs.

In the recent years, the researchers are mostly concentrating on the enhancement of protocorm like bodies (PLBs) to make synthetic seed system commercial for the propagation of orchids. Zhang et al*.* (2009) showed a protocorm suspension culture of *Cremastra appendiculata* by liquid suspension culture system and formation synthetic seeds. Production and storage of synthetic seeds of *Coelogyne breviscapa* and *Vanda coerulea* using PLBs were reported, respectively by Mohanraj et al*.* (2009) and Sarmah et al. (2010). Nagananda et al. (2011) reported the regeneration of encapsulated protocorm like bodies of *Flickingeria nodosa*.

THE GENETIC STABILITY OF SYNTHETIC SEEDS

Synthetic seeds have been widely used for micro-propagation of many plant species. The molecular studies to determine genetic stability of synthetic seeds derived plantlets were started from the last decade, but no modifications were revealed at the biochemical and/or molecular levels. The potential advantage of synthetic seeds for genetically identical to natural plants was supported by many reports (Nyende et al., 2003). The genetic stability of plantlets derived from encapsulated *Ananas comosus* micro shoots was proved by random amplified polymorphic DNA (RAPD) and ISSR techniques (Gangopadhyay et al., 2005). Bekheet (2006) reported that in *Allium sativum* both plantlets derived from encapsulated bulblets as well as normally *in vitro* were genetically similar to those that the *in vivo*. RAPD analysis showed the genetic stability of *in vitro* plantlet derived from encapsulated shoot tips of *Dioscorea bulbifera* (Narula et al., 2007). Srivastava et al (2009) reported that *Cineraria maritana*, analysis of the RAPD profiles revealed an average similarity coefficient of 0.944, they confirmed the molecular stability of plants derived from encapsulated microshoots followed by six months of storage. The genetic stability between mother plants and somatic embryo derived synthetic seeds showed resemblance in *Cucumis sativus* and proved by using RAPD markers (Tabassum et al., 2010). In *Picrorhiza kurrooa,* the genetic stability of plants derived from encapsulated microshoots following three months of storage was proved by using cluster analysis of RAPD profile (Mishra et al*.,* 2011). Lata et al. (2011) reported genetic stability of synthetic seed derived plants of *Cannabis sativa* studied by using ISSR- DAN fingerprinting and gas chromatography (GC) analysis of six major cannabinoids and showed homogeneity in the regrown clones and the mother plant.

CONCLUSION

Synthetic seeds technique is a rapid tool of plant regeneration because of its wide use in conservation and delivery of tissue cultured plants. Protocols of encapsulation were already optimized for various plant species, but the commercial scale production of synthetic seeds was restricted to few species only due to several major problems, such as: asynchronous development of somatic embryos, improper maturation of somatic embryos, poor conversion rate of somatic embryos, lack of dormancy, and limited production of viable mature somatic embryos. Such investigations need a lot of efforts to perfect this technology and to make it available on a commercial scale.

In other cases where synthetic seeds were developed by encapsulating vegetative propagules, *in vitro* raised plantlets were used as the source of explants. So, optimized micropropagation systems would be required prior to synthetic seed development. In some plant species (trees) conversion of synthetic seeds into plantlets is another major problem for commercial application. Better understanding about manipulations in the composition of synthetic endosperm, explant size, media composition, change in the formulation of medium and type of medium, optimization of growth regulators and addition of other additives to the synthetic endosperm are required to enhance the germination frequency of encapsulated propagules. However further detailed research is needed mainly for improvement in conversion frequency of synthetic seeds and subsequent plantlet growth in soil.

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Abbreviations

MS, Murashige and Skoog medium; **BA,** 6- benzyl-

adenine; **µM,** micro moles; **mg/l**, milligrams per liter; **NAA,** α- napthalene acetic acid; **GA_3**, gibberellic acid; **ABA,** absicic acid; **Synseed,** synthetic seed; **KN,** Kinetin; **TDZ,** thidiazuron; **PPM,** plant preservative mixture; **PLBs,** protocorm like bodies; **ISSR,** inter simple sequence repeat; **RAPD,** random amplified polymorphic DNA; **IBA,** indole 3 butyric acid.

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