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REVIEW

Synthetic seed production of medicinal plants: a review on influence of explants, encapsulation agent and matrix

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Abstract The present review illustrates the implementation of synthetic seed technology for mass propagation and short-term storage of several medicinal plants, popularly grown throughout the world. Biotechnology-based research with special reference to in vitro plant cell and tissue culture intervention created a new outlook in terms of mass propagation, germplasm storage and cryoconservation, production of secondary metabolites as well as genetic transformation. Synthetic seed technology involving alginate encapsulation of in vitro or in vivo generated explants proved to be a competent system to deal with multiplication, storage and exchange of seedless medicinal plants having traits of choice that are intricate to propagate via conventional approach. Nevertheless, optimization of production, storage and exchange of synthetic seeds are influenced by several factors. Manipulation of those factors such as explant selection, encapsulating agent and matrix determined the success of synthetic seed technology in medicinal plants. The present review elucidates an outline of past progress, present status and future prospects of synthetic seed technology intervention in medicinal plants with special emphasis on the factors which determine the success of this technology.

Keywords Axillary bud · Calcium chloride · Nodal segment · Shoot tip · Sodium alginate · Somatic embryo

Abbreviations

AB	Axillary bud
Ca	Calli
CS	Cell suspension
HR	Hairy root
MSt	Microshoot
NS	Nodal segment
PGR	Plant growth regulator
PLB	Protocorm-like body
SE	Somatic embryo
ST	Shoot tip

Introduction

Production of seed occurs as an outcome of a sexual procedure; on that account, in cross-pollinating species the naturally produced seeds are genetically different to individual parent (Senaratna 1992). A distinctive feature of seeds is its potential to secure dormancy by undergoing intense self-drying which allows it to be preserved for a long period of time. Nevertheless, several tropical and subtropical plants with pharmaceutical value consist of certain distinctiveness, thus making it complicated to conserve through conventional methods. A large number of medicinal plant species bear desiccation-sensitive or recalcitrant seeds that confine the storage duration only up to

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few weeks or months. Apart from that, high metabolic activities and high incidence of pathogenic infestation play significant role in reducing the storage span. Furthermore, the vulnerability of germplasm, while collecting from field gene banks, increases since they are subjected to attacks by pests and pathogens or to natural catastrophes. It is due to the vegetative nature of the germplasm that distribution and exchange of the same become inconvenient and a substantial threat of disease transmission prevails (Chaudhury and Malik 2003). Recently, an escalating interest has shown to be perceived on the usage of synthetic seed produced via encapsulation technology (Fig. 1) that is quite an impeccable route for safer conservation and exchange of the species. For the preservation and large-scale micropropagation of exclusive rare hybrids, choice genotypes and sterile unsteady genotypes, as well as genetically modified plants with non-availability of seeds or that which demands a mycorrhizal–fungal liaison for their germination, synthetic seed technology has opened new direction with huge opportunity. Of late, the interest of researchers has been focused on the encapsulation technology for the delivery of germplasm and for other assorted analytical studies (Ara et al. 2000). Although, the primary account on the formation of artificial seed was reported by Kitto and Janick (1982), the concept of the same was specified by Murashige (1977). The innovation of desiccated synthetic seeds through application of water-soluble resin coat and polyoxyethylene glycol over carrot somatic embryo (SE) was reported by Kitto and Janick (1982). Later on, Redenbaugh et al. (1984) successfully produced synthetic seeds for alfalfa through encapsulation of SEs with alginate hydrogel. Ever since, quite a number of research groups have been reported to progress work upon synthetic seeds for various plants counting cereals, fruits, ornamentals, medicinal plants, vegetables, trees and orchids (reviewed by Rai et al. 2009).

In spite of the fact that the technique for artificial seed production has been reported for more than three decades,

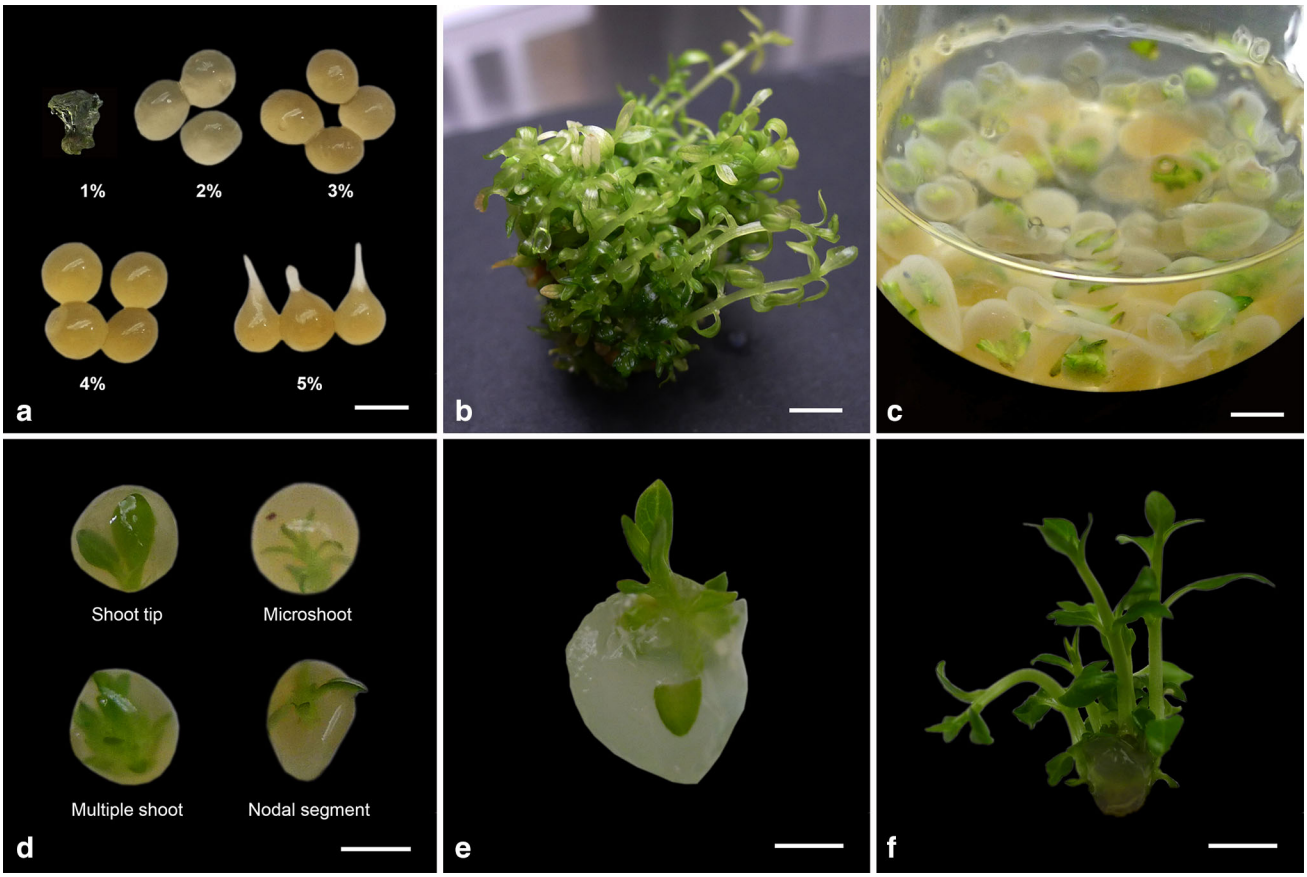

Fig. 1 Different stages of synthetic seed development and its regeneration in *Rauvolfia serpentina*, an important medicinal plant. **a** Effect of sodium alginate level (in 100 mM calcium chloride) on size, shape and consistency of beads (*bar* 5 mm), **b** in vitro-regenerated multiple shoots serving as a source of explants for encapsulation (*bar* 3 mm), **c** polymerization of encapsulated explants (*bar* 5 mm), **d** encapsulated shoot tip, microshoot, multiple shoot and nodal segment in 3 % (w/v) sodium alginate and 100 mM calcium chloride (*bar* 3 mm), **e** germinating synthetic seed developed from encapsulation of nodal segment (*bar* 2 mm), **f** germinated synthetic seed with well developed shootlets (on plant growth regulator-free MS medium) (*bar* 5 mm) (source: unpublished photos of S. Gantait and S. Kundu)

yet the same has not been developed amply for the medicinal plants. Rare and threatened medicinal plants may be safeguarded from various adversities by the implementation of encapsulated shoot tip (ST) and nodal segment (NS). The key imperfections in conventional propagation of a few species with medicinal value are: diminished endosperm, low germination rate and seedless varieties (Saiprasad 2001). Quite a number of taxa are desiccation-sensitive or possess recalcitrant seeds for which they only can be stored for a limited time (Rai et al. 2008). The exigency for the production of artificial seeds as a technique, which blends the benefits of clonal propagation with those of seed propagation and storage, can be inferred from the above-mentioned reasons. To have an edge over all these issues, considerable attention has been paid to apply encapsulated SEs in clonal propagation and short-term storage (reviewed by Kikowska and Thiem 2011).

However, there are several factors that substantially influence the success of encapsulation of plant materials to produce synthetic seeds, their storage and regeneration. The factors range from initial choice of explants or plant materials, encapsulating agent and matrix. This review gives a complete illustration of existing scenario of synthetic seed development in several plants with medicinal importance as influenced by the above-mentioned factors and their prospects in conservation and germplasm exchange. The details of the factors those play critical role in synthetic seed development and regeneration of medicinal plants are summarized in Table 1.

Selection of plant materials

The technique for production of artificial seed of medicinal plants includes encapsulation of plant material (approximately 3–5 mm long). As explants, a variety of unipolar vegetative propagules, microcuttings, differentiating aggregates or bipolar vegetative propagules such as SE and protocorm-like body (PLB) were successfully exploited, for the development of synthetic seeds. At the commencement, for artificial seed production, SEs were exploited as plant material. Substantial endeavors have achieved encapsulation of both embryogenic and non-embryogenic in vitro-derived plant material (Gantait et al. 2012; Gantait and Sinniah 2013), in general, over the past several years (as reviewed by Ara et al. 2000; Lambardi et al. 2006; Sharma et al. 2013). However, the present review gives updated accounts chiefly since last two decades on selection of planting materials and their influence on encapsulation of typical medicinal plants, in particular (Table 1).

Somatic embryos

The mechanism by virtue of whichever haploid or diploid cells turn to plants, experiencing distinctive embryonic phases, devoid of the union of gametes, is stated as somatic embryogenesis (Williams and Maheswaran 1986). The procedure may possibly be direct with the direct development of embryogenic cells from explanted cells, or else indirect with a transitional callogenesis period (Merkle et al. 1990). Since an SE has the competence to possess shoot and root poles simultaneously, it is considered to be a bipolar structure (Standardi and Piccioni 1998). It is because of its bipolar nature that amidst a range of assorted propagules, SE has been triumphant in establishing the fact that it is the best suited entity for synthetic seed production. Since, SEs possess the efficiency to be formed clonally in bulk, that makes them desirable items for encapsulation. Synthetic seeds, comprising SE surrounded in a defensive covering, was suggested as a low-cost, high-capacity propagation technique (Redenbaugh 1990). Keeping in view the remarkable advantages that SEs possess over auxiliary propagules the same was fruitfully exercised for synthetic seed development in scores of plant species. The prospective and employment of SE in development of synthetic seeds has been reviewed and presented in Table 1. SE was effectively utilized for synthetic seed production in several medicinal plant species, for instance, *Musa* spp. (Ganapathi et al. 2001), *Quercus robur* (Prewein and Wilhelm 2003), *Rotula* aquatic (Chithra et al. 2005); *Pinus patula* (Malabadi and van Staden 2005), *Arnebia euchroma* (Manjkhola et al. 2005); *Citrus nobilis* × *C. deliciosa* (Singh et al. 2007), *Vitis vinifera* (Nirala et al. 2010), *Dalbergia sissoo* (Singh and Chand 2010), *Clitoria ternatea* (Kumar and Thomas 2012), *Catharanthus roseus* (Maqsood et al. 2012), *Hemidesmus indicus* (Cheruvathur et al. 2013a), *Rhinacanthus nasutus* (Cheruvathur et al. 2013b), *Artemisia vulgaris* L. (Sudarshana et al. 2013), *Anethum graveolens* (Dhir et al. 2014), *Swertia chirayita* (Kumar and Chandra 2014).

It was evident that there were two major hurdles to overcome in the way of producing artificial seeds using SE as explants. In the procedure involving utilization of SE for synthetic seed production, the major impediment is a metachronus and delayed development of the embryonic terminal (Castellanos et al. 2004). Even though the encapsulation phase was achieved fruitfully, yet the regeneration of encapsulated SE was not successful in comparison to the other explants. The regeneration was around 50 % in majority of the medicinal plants taken into account. It is significant to mention, there was only 26 % regeneration in case of *Quercus robur* synthetic seeds cultured on P24 medium fortified with 0.9 μM BA and

Table 1 Artificial seed production at their optimal conditions for different medicinal plant species (in chronological order and alphabetical order within each year)

Species	Explant type	Sodium alginate (%)	Conc. of calcium chloride (mM)	Regeneration (%)	References
Daucus carota L.	SE	3	100	66	Kamada et al. (1989)
Santalum album L.	SE	3	62.7	–	Bapat and Rao (1992)
Asparagus cooperi Baker	SE	3.5	50	32.3	Ghosh and Sen (1994)
Camellia japonica	SE	3	100	63	Janeiro et al. (1997)
Armoracia rusticana	HR	2	50	85	Phunchindawan et al. (1997)
Actinidia deliciosa	AB	2.5	100	50–57	Adriani et al. (2000)
Ocimum sp.	ST	4	75	95–99	Mandal et al. (2000)
Morus sp.	NS, AB	4	75	78–98	Pattnaik and Chand (2000)
Musa spp. AAB group	SE	5	–	66	Ganapathi et al. (2001)
Adhatoda vasica	ST	4	100	66.28	Anand and Bansal (2002)
Allium sativum	Ca	1.5	50	95	Kim and Park (2002)
Quercus robur	SE	4	50	26	Prewein and Wilhelm (2003)
Dalbergia sissoo	NS	3	75	85	Chand and Singh (2004)
Rotula aquatic	SE	3	50	100	Chithra et al. (2005)
Ananus comosus	NS	2.5	–	86.13	Gangopadhyay et al. (2005)
Pinus patula	SE	2.5	100	89	Malabadi and van Staden (2005)
Arnebia euchroma	SE	–	25	60.6	Manjkhola et al. (2005)
Rhodiola kirilowii	AB, Ca	5	50	100	Zych et al. (2005)
Dendranthema × *grandiflora*	NS	3	75	50	Pinker and Abdel-Rahman (2005)
Morus sp.	AB	4	50	48.2	Kavyashree et al. (2006)
Punica granatum	NS	3	100	75	Naik and Chand (2006)
Chonemorpha grandiflora	ST	3	50	95	Nishitha et al. (2006)
Phyllanthus amarus	ST	3	75	90	Singh et al. (2006a)
Withania somnifera (L.) Dunal	ST	3	75	87	Singh et al. (2006b)
Populus tremula × *P. tremuloides*	ST	4	1.4	100	Tsvetkov et al. (2006)
Tylophora indica	NS	3	100	91	Faisal and Anis (2007)
Olea europaea	NS	2.5	100	–	Micheli et al. (2007)
Citrus nobilis × *C. deliciosa*	SE	4	75	81.94	Singh et al. (2007)
Coleus forskohlii	ST, NS	3	90	92	Swaroopa et al. (2007)
Rhododendron maddeni	ST	3	60	96	Singh (2008)
Pogostemon cablin Benth.	NS	4	100	73.3	Kumara Swamy et al. (2009)
Cannabis sativa	NS	5	50	100	Lata et al. (2009)
Curculigo orchioides	ST	2.5	100	68	Nagesh et al. (2009)
Spilanthes mauritiana	NS	4	100	>90	Sharma et al. (2009a)
Spilanthes acmella	NS	4	100	87.8	Sharma et al. (2009b)
Ocimum basilicum	NS	3	75	80	Siddique and Anis (2009)
Spilanthes acmella	ST	3	100	100	Singh et al. (2009)
Cineraria maritima	ST, NS	3	272.7	11.76	Srivastava et al. (2009)
Pogostemon cablin	NS	4	100	91.1	Swamy et al. (2009)
Vitex negundo	NS	3	100	92.6	Ahmad and Anis (2010)
Simmondsia chinensis	ST	3	100	66.6	Kumar et al. (2010)
Vitis vinifera	SE	2	100	36	Nirala et al. (2010)
Eclipta alba	ST, NS	3	100	94.3	Ray and Bhattacharya (2010)
Eclipta alba	NS	3	100	100	Singh et al. (2010)
Dalbergia sissoo	SE	2.5	43.3	43.3	Singh and Chand (2010)

Table 1 continued

Species	Explant type	Sodium alginate (%)	Conc. of calcium chloride (mM)	Regeneration (%)	References
Zingiber officinale	MSs	4	100	66	Sundararaj et al. (2010)
Cucumis sativus	CS	3	100	57	Tabassum et al. (2010)
Citrus sinensis × *Poncirius trifoliate*	NS	2.5	100	100	Germanà et al. (2011)
Khaya senegalensis	ST, NS	3	100	52–98	Hung and Trueman (2011)
Picrorhiza kurrooa	ST, NS	3	272.7	21.43	Mishra et al. (2011)
Rauvolfia tetraphylla	NS	3	100	90.3	Alatar and Faisal (2012)
Stevia rebaudiana	ST, NS	3	100	100	Ali et al. (2012)
Rauvolfia serpentina	NS	3	100	80	Faisal et al. (2012)
Corymbia torellina × *C. citriodora*	ST, NS	3	100	62–100	Hung and Trueman (2012a, 2012b)
Clitoria ternatea	SE	4	100	92	Kumar and Thomas (2012)
Cannabis sativa	NS	5	50	60	Lata et al. (2012)
Catharanthus roseus	SE	2.5	100	84.33	Maqsood et al. (2012)
Decalepis hamiltonii	NS	4	100	77	Sharma and Shahzad (2012)
Ruta graveolens	NS	3	100	86.7	Ahmad et al. (2012)
Curcuma amada	MSt	3	–	75	Banerjee et al. (2012)
Mentha arvensis	ST, NS	–	–	80	Islam and Bari (2012)
Beta vulgaris	ST	4	100	–	Rizkalla et al. (2012)
Hemidesmus indicus	SE	3	75	100	Cheruvathur et al. (2013a)
Rhinacanthus nasutus	SE	3	100	94	Cheruvathur et al. (2013b)
Ceropegia bulbosa	NS	3	100	100	Dhir and Shekhawat (2013)
Rauvolfia tetraphylla	NS	3	100	80.6	Faisal et al. (2013)
Withania somnifera	NS	3	100	86.2	Fatima et al. (2013)
Stevia rebaudiana	NS	4	75	60–70	Khan et al. (2013)
Dendrobium nobile	PLB	3	100	78.2	Mohanty et al. (2013)
Ceropegia spiralis, *C. pusilla*	ST, NS	1–5	25–100	86.6–90	Murthy et al. (2013)
Centaurium erythraea	ST, HR	3	50	86	Piątczak and Wysokińska (2013)
Picrorhiza kurrooa	HR	–	–	73	Rawat et al. (2013)
Aristolochia tagala	MSt	3	68	80	Remya et al. (2013)
Begonia semperflorens	MSt	3	100	–	Sakhanokho et al. (2013)
Artemisia vulgaris L.	SE	2	75	90	Sudarshana et al. (2013)
Cucumis sativus	ST	3	100	–	Adhikari et al. (2014)
Ochradenus baccatus	–	3	100	86	Al-Qurainy et al. (2014)
Tuberaria major	ST	3	75	76.7	Coelho et al. (2014)
Anethum graveolens	SE	3	100	83	Dhir et al. (2014)
Terminalia arjuna	ST	3	100	91.6	Gupta et al. (2014)
Swertia chirayita	SE	4	100	84	Kumar and Chandra (2014)
Stevia rebaudiana	ST	4	100	69	Nower (2014)
Centella asiatica	AB, NS	4	75	85.7	Prasad et al. (2014)
Cassia angustifolia Vahl.	NS	3	100	94	Parveen and Shahzad (2014)
Ocimum gratissimum	MSt	3	75	98.62	Saha et al. (2014a)
O. kilimandscharicum	ST	3	75	79.53	Saha et al. (2014b)
Salvia splendens	NS	4	100	63.6	Sharma et al. (2014)
Sterculia urens	NS	4	100	95	Subhashini Devi et al. (2014)
Phyllanthus fraternus	NS	3	100	92.5	Upadhyay et al. (2014)
Balanites aegyptiaca	NS	3	100	80	Varshney and Anis (2014)

Table 1 continued

Species	Explant type	Sodium alginate (%)	Conc. of calcium chloride (mM)	Regeneration (%)	References
Vitex trifolia L.	NS	3	100	84.9	Ahmed et al. (2015)
Tecomella undulata	NS	3	100	58.5	Shaheen and Shahzad (2015)

- denotes unavailability of information

AB axillary bud, *Ca* calli, *CS* cell suspension, *HR* hairy root, *MSt* microshoot, *NS* nodal segment, *PLB* protocorm-like body, *SE* somatic embryo, *ST* shoot tip

0.1 μM IBA (Prewein and Wilhelm 2003). There was only one instance where synthetic seeds developed from SE resulted in 100 % regeneration in MS with 2 μM BA plus 0.5 μM IBA (in *Hemidesmus indicus* by Cheruvathur et al. 2013a). Interestingly, both of these extreme results were achieved in comparable media formulations which explain the species specificity of SE in terms of its regeneration potency. Hence, to improve plantlet conversion from synthetic seeds, an adept embryogenic system is indispensible. In terms of synthetic seeds, empirical use of somatic embryogenesis has not yet evolved accordingly. Within a species, embryogenic competency is frequently in a small number of genotypes, or is attained either by employing zygotic embryos or immature explants. This restricts its exercise for propagating preferred cultivars (Standardi and Piccioni 1998). Furthermore, in spite of expensive production procedure, yet there prevails a low success on encapsulation potency of SE, buds, shoots or other meristematic tissue that can develop into a complete plant and thus can be considered as 'artificial seeds' (Pond and Cameron 2003).

Shoot tips

Amid various other non-embryogenic materials, ST explants have proven to be the most amenable on account of their mitotic activity in the meristem (Ballester et al. 1997). STs possessing apical buds were utilized for the encapsulation of some medicinal plant species (Table 1). The most interesting part of using shoot buds as explants is the requirement of space and cost of mass propagation via synthetic seed production in comparison to conventional ST culture in vitro. The amount of space and culture media needed for multiple shoot formation and its proliferation is 20 times higher than what is required for micropropagation through synthetic seeds (Fig. 1b, c), using shoot buds as explants. Therefore, encapsulation of shoot buds (apical or axial) provides for an effortless transportation of considerably bulk number of propagules even in limited space (Fig. 1c). Apical and axial shoot buds can easily be regenerated into plants, as long as there is an ample supply of nutrients and rooting does not pose to be problematic; sometimes these regenerate spontaneously from synthetic seeds (Fig. 1d, e). Moreover, further investigation is necessary to determine the procedure by which the growth of apical and axial buds, in an encapsulation matrix, can be suspended until desirable span of time.

STs were effectively utilized for synthetic seed production (Fig. 1d) in several medicinal plant species, such as, *Adhatoda vasica* (Anand and Bansal 2002), *Chonemorpha grandiflora* (Nishitha et al. 2006), *Phyllanthus amarus*, *Withania somnifera* (Singh et al. 2006a, b), *Populus tremula* × *P. tremuloides* (Tsvetkov et al. 2006), *Coleus forskohlii* (Swaroopa et al. 2007), *Rhododendron maddeni* (Singh 2008), *Curculigo orchioides* (Nagesh et al. 2009), *Cineraria maritime* (Srivastava et al. 2009), *Simmondsia chinensis* (Kumar et al. 2010), *Eclipta alba* (Ray and Bhattacharya 2010), *Khaya senegalensis* (Hung and Trueman 2011), *Picrorhiza kurrooa* (Mishra et al. 2011), *Stevia rebaudiana* (Ali et al. 2012), *Corymbia torellina* × *C. citriodora* (Hung and Trueman 2012a, b), *Mentha arvensis* (Islam and Bari 2012), *Beta vulgaris* (Rizkalla et al. 2012), *Ceropegia spiralis*, *C. pusilla* (Murthy et al. 2013), *Centaurium erythraea* (Piątczak and Wysokińska 2013), *Cucumis sativus* (Adhikari et al. 2014), *Terminalia arjuna* (Gupta et al. 2014), *Stevia rebaudiana* (Nower 2014).

Nodal segments

Most commonly used propagules, denoted to as microcuttings (viz. NS), are used for synthetic seed production, most likely because of the fact that these explants are produced with such relative simplicity, after the micropropagation technique has been set up (Piccioni and Standardi 1995). In terms of storage potential, the period sufficient for exchange between laboratories and the post-storage proliferation with rooting abilities, encapsulated microcuttings proved to be efficient without the risk of physiological variation (Micheli et al. 2007). NSs with axillary buds (ABs) were effectively utilized for synthetic seed production (Fig. 1d) in several medicinal plant species, such as, *Morus* sp. (Pattnaik and Chand 2000), *Dalbergia sissoo* (Chand and Singh 2004), *Ananus comosus*

(Gangopadhyay et al. 2005), *Dendranthema* × *grandiflora* (Pinker and Abdel-Rahman 2005), *Punica granatum* (Naik and Chand 2006) *Tylophora indica* (Faisal and Anis 2007), *Olea europaea* (Micheli et al. 2007), *Coleus forskohlii* (Swaroopa et al. 2007), *Cannabis sativa* (Lata et al. 2009), *Spilanthes mauritiana* (Sharma et al. 2009a), *Spilanthes acmella* (Sharma et al. 2009b), *Ocimum basilicum* (Siddique and Anis 2009), *Cineraria maritime* (Srivastava et al. 2009), *Pogostemon cablin* (Swamy et al. 2009), *Vitex negundo* (Ahmad and Anis 2010), *Eclipta alba* (Ray and Bhattacharya 2010), *Eclipta alba* (Singh et al. 2010), *Citrus sinensis* × *Poncirius trifoliate* (Germanà et al. 2011), *Khaya senegalensis* (Hung and Trueman 2011), *Picrorhiza kurrooa* (Mishra et al. 2011), *Rauvolfia tetraphylla* (Alatar and Faisal 2012), *Stevia rebaudiana* (Ali et al. 2012), *Rauvolfia serpentine* (Faisal et al. 2012), *Corymbia torellina* × *C. citriodora* (Hung and Trueman 2012a, b), *Cannabis sativa* (Lata et al. 2012), *Decalepis hamiltonii* (Sharma and Shahzad 2012), *Ruta graveolens* (Ahmad et al. 2012), *Curcuma amada* (Banerjee et al. 2012), *Mentha arvensis* (Islam and Bari 2012), *Ceropegia bulbosa* (Dhir and Shekhawat 2013), *Rauvolfia tetraphylla* (Faisal et al. 2013) *Withania somnifera* (Fatima et al. 2013), *Stevia rebaudiana* (Khan et al. 2013), *Ceropegia spiralis*, *C. pusilla* (Murthy et al. 2013), *Centella asiatica* (Prasad et al. 2014), *Salvia splendens* (Sharma et al. 2014), *Sterculia urens* (Subhashini Devi et al. 2014), *Phyllanthus fraternus* (Upadhyay et al. 2014), *Balanites aegyptiaca* (Varshney and Anis 2014). It was observed from these studies that there was emergence of multiple shoots frequenting from the synthetic seeds (Fig. 1f), thus proving the encapsulation of NSs as a proficient approach and signifying the occurrence of multiple axillary primordia on the explants.

Protocorm-like bodies

With an exception in atypical instances (Ilan et al. 1995), the term 'protocorm-like body' remained limited to in vitro culture of orchids (Ishii et al. 1998). The structures of PLBs were differentiated from typical embryos by the absence of a distinct embryonic alignment (Norstog 1979). Alternatively they are formed with several meristematic centers that modify into regular embryos, shoots and roots (da Silva et al. 2000). Generally, orchid breeders sprout thousands of seeds in tiny containers, within sterile environment. The resultant protocorms have a tendency to develop into bunches of seedlings that is required to be detached physically; a process which is both tiresome and expensive. The breakthrough achieved by the innovation of synthetic seed system wherein single seed or protocorm were encapsulated in an apposite matrix, would evidently minimize the intricacy of seedling sorting and planting. Likewise, for the purpose of encapsulation, PLBs could be employed. So far, the only study involving PLBs as explants to produce synthetic seeds of medicinal plants was carried out by Mohanty et al. (2013) in *Dendrobium nobile*, a typical orchid with pharmaceutical importance. Utilizing the direct regeneration potential of PLBs, the same achieved near 80 % conversion of synthetic seeds. PLBs are proliferated asexually and the synthetic seeds prepared thereof can ideally be termed as clonal seeds, provided they retain their genetic fidelity.

Hairy roots

Inoculating the plants with *Agrobacterium rhizogenes* triggers the transfer-DNA comprised in the plasmid to be introduced in plant genome and simultaneously promotes the induction of 'hairy roots' that promptly advances rapid growth of root cultures. Such hairy root cultures are regarded as valuable resources of secondary metabolites and enzymes. Circumventing the extensive use of conventional unipolar and bipolar explants, Piątczak and Wysokińska (2013) and Rawat et al. (2013) employed hairy root fragments as a novel approach in synthetic seed development of medicinal plants *Centaurium erythraea* and *Picrorhiza kurrooa* with 86 and 73 % regeneration, respectively. Eventually they extended the conception of synthetic seeds by introducing fragments of hairy roots for the first time in medicinal plants.

Calli

Among a wide array of propagules, calli is the least exploited in terms of synthetic seed production of medicinal plants, based on its intricate use during encapsulation and subsequent regeneration. The undifferentiated nature of calli and the requirement of successful differentiation potential presumably restrict its acceptance as an explant for synthetic seed production. Kim and Park (2002), and later on Zych et al. (2005) were the only researchers who successfully encapsulated calli of *Allium sativum* and *Rhodiola kirilowii*, respectively, and attained as high as 95–100 % regeneration. However, in a more recent study on *Cucumis sativus*, Tabassum et al. (2010) employed cell suspension derived from friable callus and ensured 57 % regeneration of synthetic seeds.

Encapsulating agent and matrix

Since the matrix of capsules is largely accountable for the direct surroundings of the plant material and thus proves that the same has a significant impact on the eventual sustainability of the synthetic seed. It is absolutely

necessary that the artificial seed coat ought to shield the explants, possess the efficiency to include nutrients as well as other growth and biological factors, protect the developed artificial seed throughout storage and handling, subsume a mechanism for activating 'germination', be innocuous, maintain a good affinity with the biological and chemical systems; and lastly, should rather be biodegradable as well (Khor and Loh 2005). The soft hydrogel protects the explant by making sure that the least amount of pressure be put on the same, thus assuring a much lesser damage to the plant material. In the perspective of hydrogel, the explants are commingled with a polymeric solution that when dropped in the other liquid comprising divalent metal ions, commenced a cross-linking effect, giving rise to the hydrogel. The resultant encapsulation beads held the explants firmly and continued to provide sufficient resistance to exterior mechanical stress, for easy handling.

It is quite evident, notwithstanding the fact, that the selection of plant material apposite for propagation into a plant is the nucleus of the synthetic seed conception; the selection of associated matrix constituents employed in combination with the biological substances is equally vital. Quite a few factors are responsible for the successful production of synthetic seeds, counting the crucial ones such as the level and type of gel required for encapsulation and the extent of exposure of encapsulated seeds to $CaCl_2 \cdot H_2O$, as well (Redenbaugh et al. 1991, 1993). Gels such as agar, alginate, carboxy methyl cellulose, carrageenan, gelrite, sodium pectate, etc., were exploited for artificial seed development, of which alginate encapsulation was proved to be appropriate for the same. Several gel types are exploited for encapsulation; however, sodium alginate established itself to be the most frequently used matrix because of low cost, gelling properties, and its nontoxic nature (Cheruvathur et al. 2013a). Mainly, the hardness of the hydrogels is contingent on the number of Na^+ ions (in sodium alginate solution), exchanged with Ca^{2+} ions (in $CaCl_2 \cdot H_2O$ solution), consequently ensuing in the creation of insoluble calcium alginate (Daud et al. 2008). Usage of agar as gel matrix is not preferred because of its inferior nature when compared to alginate relating to long-standing storage. Alginate is the preferred choice since it aids to ameliorate capsule development and additionally, the firmness of alginate beads assures a much-improved protection (in comparison to agar) to the encased somatic embryos from mechanical damage (Saiprasad and Polisetty 2003).

Several aspects come into play in moderating the environmental elements of temperature and humidity in order to safeguard the biological substances, and additionally offering a sizeable nutrient pool. Evidently, it can be said that matrix materials play a crucial role in the evaluation of the definitive sustainability of synthetic seed. Over the times, the conception of matrix materials has evolved into a moderately refined interaction that centers on the transformability of synthetic seeds. It was evident from the present review update that the majority of the optimum results in terms of nicely formed spherical beads were obtained with 100 mM $CaCl_2 \cdot H_2O$ and 3 % sodium alginate, for example, in *Punica granatum* (Naik and Chand 2006), *Tylophora indica* (Faisal and Anis 2007), *Vitex negundo* (Ahmad and Anis 2010), *Simmondsia chinensis* (Kumar et al. 2010), *Eclipta alba* (Ray and Bhattacharya 2010, and Singh et al. 2010), *Cucumis sativus* (Tabassum et al. 2010), *Khaya senegalensis* (Hung and Trueman 2011), *Rauvolfia tetraphylla* (Alatar and Faisal 2012), *Stevia rebaudiana* (Ali et al. 2012), *Rauvolfia serpentine* (Faisal et al. 2012), *Corymbia torellina* × *C. citriodora* (Hung and Trueman 2012a, b), *Ruta graveolens* (Ahmad et al. 2012), *Rhinacanthus nasutus* (Cheruvathur et al. 2013b), *Ceropegia bulbosa* (Dhir and Shekhawat 2013), *Rauvolfia tetraphylla* (Faisal et al. 2013), *Withania somnifera* (Fatima et al. 2013), *Dendrobium nobile* (Mohanty et al. 2013), *Begonia semperflorens* (Sakhanokho et al. 2013), *Cucumis sativus* (Adhikari et al. 2014), *Ochradenus baccatus* (Al-Qurainy et al. 2014), *Anethum graveolens* (Dhir et al. 2014), *Terminalia arjuna* (Gupta et al. 2014), *Phyllanthus fraternus* (Upadhyay et al. 2014), and *Balanites aegyptiaca* (Varshney and Anis 2014), and the regeneration of synthetic seeds was around or more than 90 % in majority of the instances. Seemingly, 3 % solution of sodium alginate and 100 mM $CaCl_2$ facilitated in the most advantageous ion exchange involving Na^+ and Ca^{2+}, producing compact, transparent, isodiametric beads. Lesser concentrations (1 and 2 %) of sodium alginate were not apposite in view of the fact that the capsules were of asymmetrical shape and being exceedingly fragile and squashy to hold; at the same time at elevated levels (4 and 5 % sodium alginate) the capsules were effectively firm (Fig. 1a) resulting in considerable impediment in conversion of synthetic seeds (in terms of emergence of propagule through breaking the alginate coat). The instances mentioned above were based on assessing the function of sodium alginate level, influencing the gel matrix or bead attribute and successive transformation of synthetic seeds. Reduced levels of sodium alginate may trigger lower endurance of beads formed. The lower endurance of beads exposed for a longer phase (usually for 30 min) to $CaCl_2$ could adsorb large amounts of $CaCl_2$ (Nagesh et al. 2009). Since adsorption is a surface phenomenon, higher accumulation of $CaCl_2$ might restrain further progress, consequently initiating $CaCl_2$-toxicity (Nagesh et al. 2009). On the contrary, beads with greater resistance formed because of higher level of sodium alginate (3 %) could adsorb fewer amounts of $CaCl_2$ and thus ensuing lesser toxicity and ultimately resulting in high-frequency recuperation.

Singh et al. (2010) also presented a report on the impact of sodium alginate and $CaCl_2$ amalgamations on bead texture and uniformity, in *Eclipta alba* (L.) Hassk, one of the leading medicinal herbs. A comprehensive study has been carried out on sodium alginate. It is a biodegradable and biocompatible copolymer containing L-glucuronic and D-mannuronic acid units, and possesses the capability to produce hydrogels in presence of divalent cations. The taut structure and oversized pores of these water-insoluble gels make them proficient enough for encapsulation of live plant cells (Bajaj 1995). Moreover, these allow the exchange of elements back and forth from the adjoining medium.

Concluding remarks

From the available research literatures it is established that the explant-types and concentrations of encapsulation agent as well as matrix have noteworthy roles in production of synthetic seeds in medicinal plants. These three factors chiefly determine the fate of synthetic seeds escalating their conversion in a number of ways. Exploitation of unipolar explants such as shoot tip, nodal segment with axillary bud, microshoots, etc., with high morphogenic potential are the most responsive throughout the synthetic seed development procedure. However, the promoter role of these unipolar explants further depends on several other factors those influencing the regeneration prospective. Optimal levels of PGRs and their types in the conversion media regulate the germination of synthetic seeds and their subsequent development into complete plantlets. A coating of alginate, which provides ideal protection to the juvenile tissue inside and simultaneously facilitating the easy breakage during germination as and when required, is of utmost importance, apart from the standard media for conversion. Nonetheless, few negative reports exist as well. For instance, Tabassum et al. (2010) achieved a merely 57 % regeneration of synthetic seeds in *Cucumis sativus* using 3 % sodium alginate and 100 mM $CaCl_2{\cdot}2H_2O$ but they used cell suspension as the explant. From the existing literature it becomes further clear that in several cases the increased concentration (4 %) of sodium alginate and reduced level (75 mM) of $CaCl_2{\cdot}2H_2O$ resulted in significant conversion (95–99 %) of synthetic seeds that is comparable to that of the optimal combinations discussed earlier. Consequently, it is indispensable to focus on additional factors influencing the system of synthetic seeds formation directly or indirectly.

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References

Adhikari S, Bandyopadhyay TK, Ghosha P (2014) Assessment of genetic stability of *Cucumis sativus* L. regenerated from encapsulated shoot tips. Sci Hortic 170:115–122

Adriani M, Piccioni E, Standardi A (2000) Effect of different treatments on conversion of 'Hayward' kiwifruit synthetic seeds to whole plants following encapsulation of in vitro-derived buds. N Z J Crop Hortic Sci 28:59–67

Ahmad N, Anis M (2010) Direct plant regeneration from encapsulated nodal segments of *Vitex negundo*. Biol Plant 54:748–752

Ahmad N, Faisal M, Fatima N, Anis M (2012) Encapsulation of microcuttings for propagation and short-term preservation in *Ruta graveolens* L.: a plant with high medicinal value. Acta Physiol Plant 34:2303–2310

Ahmed MR, Anis M, Al-Etta HA (2015) Encapsulation technology for short-term storage and germplasm exchange of *Vitex trifolia* L. Rend Fis Acc Lincei. doi:10.1007/s12210-014-0366-1

Alatar A, Faisal M (2012) Encapsulation of *Rauvolfia tetraphylla* microshoots as artificial seeds and evaluation of genetic fidelity using RAPD and ISSR markers. J Med Plant Res 6:1367–1374

Ali A, Gull I, Majid A, Saleem A, Naz S, Naveed NH (2012) In vitro conservation and production of vigorous and desiccate tolerant synthetic seeds in *Stevia rebaudiana*. J Med Plant Res 6:1327–1333

Al-Qurainy F, Nadeem M, Khan S, Alansi S, Tarroum M, Al-Ameri A (2014) Synseed production for storage and conservation of *Ochradenus baccatus* delile. Pak J Bot 46:897–902

Anand Y, Bansal YK (2002) Synthetic seeds: a novel approach of in vitro plantlet formation in vasaka (*Adhatoda vascia* Nees.). Plant Biotechnol J 19:159–162

Ara H, Jaiswal U, Jaiswal VS (2000) Synthetic seed: prospects and limitations. Curr Sci 78:1438–1444

Bajaj YPS (1995) Somatic embryogenesis and synthetic seed I. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 30. Springer, Berlin

Ballester A, Janeiro LV, Vieitez AM (1997) Cold storage of shoot cultures and alginate encapsulation of shoot tips of *Camellia japonica* L. and *Camellia reticulate* Lindly. Sci Hortic 7:67–78

Banerjee S, Singh S, Pandey H, Pandey P, Rahman LU (2012) Conservation and storage of *Curcuma amada* Roxb. synseeds on Luffa sponge matrix and RAPD analysis of the converted plantlets. Ind Crops Prod 36:383–388

Bapat VA, Rao PS (1992) Plantlet regeneration from encapsulated and non-encapsulated desiccated somatic embryos of a forest tree: sandalwood (*Santalum album* L.). J Plant Biochem Biotechnol 1:109–113

Castellanos H, Sánchez-Olate M, Ríos YD (2004) Embriogénesis somátic recurrente en raulí (*Nothofagus alpine* (Poepp. et Endl.) Oerst). Segundo Congreso Chileno de Ciencias Forestales,

Valdivia, Chile. 10–12 de noviembre. Universidad Austral de Chile, Valdivia, p 36
Chand S, Singh AK (2004) Plant regeneration from encapsulated nodal segments of *Dalbergia sissoo* Roxb.—a timber yielding leguminous tree. J Plant Physiol 161:237–243
Chaudhury R, Malik SK (2003) Strategies for achieving short-, medium- and long-term conservation of desiccation-sensitive seeds. In: Chaudhury R, Pandey R, Malik SK, Bhag M (eds) In vitro conservation and cryopreservation of tropical fruit species. IPGRI Office for South Asia and NBPGR, New Delhi, pp 191–200
Cheruvathur MK, Kumar GK, Thomas TD (2013a) Somatic embryogenesis and synthetic seed production in *Rhinacanthus nasutus* (L.) Kurz. Plant Cell Tiss Organ Cult 113:63–71
Cheruvathur MK, Najeeb N, Thomas TD (2013b) In vitro propagation and conservation of Indian sarsaparilla, *Hemidesmus indicus* L. R. Br. through somatic embryogenesis and synthetic seed production. Acta Physiol Plant 35:771–779
Chithra M, Martin KP, Sunandakumari C, Madhusoodan PV (2005) Somatic embryogenesis, encapsulation, and plant regeneration of *Rotula aquatica* Lour. a rare rhoeophytic woody medicinal plant. In Vitro Cell Dev Biol-Plant 41:28–31
Coelho N, González-Benito ME, Romano A (2014) Cryopreservation of shoot tips from the endangered endemic species *Tuberaria major*. Acta Physiol Plant 36:3333–3336
da Silva ALS, Moraes-Fernandes MI, Fereira AG (2000) Ontogenetic events in androgenesis of Brazilian barley genotypes. Rev Braz Biol 60:315–319
Daud N, Taha RM, Hasbullah NA (2008) Artificial seed production from encapsulated micro shoots of *Saintpaulia ionantha* Wendl. (African Violet). J Appl Sci 8:4662–4667
Dhir R, Shekhawat GS (2013) Production, storability and morphogenic response of alginate encapsulated axillary meristems and genetic fidelity evaluation of in vitro regenerated *Ceropegia bulbosa*: a pharmaceutically important threatened plant species. Ind Crops Prod 47:139–144
Dhir R, Shekhawat GS, Alam A (2014) Improved protocol for somatic embryogenesis and calcium alginate encapsulation in *Anethum graveolens* L.: a medicinal herb. Appl Biochem Biotechnol 173:2267–2278
Faisal M, Anis M (2007) Regeneration of plants from alginate-encapsulated shoots of *Tylophora indica* (Burm. F.) Merrill., an endangered medicinal plant. J Hortic Sci Biotechnol 82:351–354
Faisal M, Alatar AA, Ahmad N, Anis M, Hegazy AK (2012) Assessment of genetic fidelity in *Rauvolfia serpentina* plantlets grown from synthetic (encapsulated) seeds following in vitro storage at 4 °C. Molecule 17:5050–5061
Faisal M, Alatar AA, Hegazy AK (2013) Molecular and biochemical characterization in *Rauvolfia tetraphylla* plantlets grown from synthetic seeds following in vitro cold storage. Appl Biochem Biotechnol 169:408–417
Fatima N, Ahmad N, Anis M, Ahmad I (2013) An improved in vitro encapsulation protocol, biochemical analysis and genetic integrity using DNA based molecular markers in regenerated plants of *Withania somnifera* L. Ind Crops Prod 50:468–477
Ganapathi TR, Srinivas L, Suprasanna P, Bapat VA (2001) Regeneration of plants from alginate-encapsulated somatic embryos of banana cv. rasthali (*Musa* spp. AAB Group). In Vitro Cell Dev Biol-Plant 37:178–181
Gangopadhyay G, Bandyopadhyay T, Ramit P, Gangopadhyay SB, Mukherjee KK (2005) Encapsulation of pineapple microshoots in alginate beads for temporary storage. Curr Sci 88:972–977
Gantait S, Sinniah UR (2013) Storability, post-storage conversion and genetic stability assessment of alginate-encapsulated shoot tips of monopodial orchid hybrid *Aranda* Wan Chark Kuan 'Blue' × *Vanda coerulea* Grifft. ex. Lindl. Plant Biotechnol Rep 7:257–266
Gantait S, Bustam S, Sinniah UR (2012) Alginate-encapsulation, short-term storage and plant regeneration from protocorm-like bodies of *Aranda* Wan Chark Kuan 'Blue' × *Vanda coerulea* Grifft. ex. Lindl. (Orchidaceae). Plant Growth Regul 68:303–311
Germanà MA, Micheli M, Chiancone B, Macaluso L, Standardi A (2011) Organogenesis and encapsulation of in vitro-derived propagules of Carrizo citrange [(*Citrus sinesis* (L.) Osb. × *Poncirius trifoliate* (L.) Raf.]. Plant Cell Tiss Organ Cult 106:299–307
Ghosh B, Sen S (1994) Plant regeneration from alginate encapsulated somatic embryos of *Asparagus cooperi* Baker. Plant Cell Rep 13:381–385
Gupta AK, Harish Manoj, Rai Phulwaria M, Agarwal T, Shekhawat NS (2014) In vitro propagation, encapsulation, and genetic fidelity analysis of *Terminalia arjuna*: a cardioprotective medicinal tree. Appl Biochem Biotechnol 173:1481–1494
Hung CD, Trueman SJ (2011) Encapsulation technology for short-term preservation and germplasm distribution of the African mahogany *Khaya senegalensis*. Plant Cell Tiss Organ Cult 107:397–405
Hung CD, Trueman SJ (2012a) Alginate encapsulation of shoot tips and nodal segments for short-term storage and distribution of the eucalypt *Corymbia torelliana* × *C. citriodora*. Acta Physiol Plant 34:117–128
Hung CD, Trueman SJ (2012b) Preservation of encapsulated shoot tips and nodes of the tropical hardwoods *Corymbia torelliana* × *C. citriodora* and *Khaya senegalensis*. Plant Cell Tiss Organ Cult 109:341–352
Ilan A, Ziv M, Halevy AH (1995) Propagation and corm development of *Brodiaea* in liquid cultures. Sci Hortic 63:101–112
Ishii Y, Takamura T, Goi M, Tanaka M (1998) Callus induction and somatic embryogenesis of *Phalaenopsis*. Plant Cell Rep 17:446–450
Islam MS, Bari MA (2012) In vitro regeneration protocol for artificial seed production in an important medicinal plant *Mentha arvensis* L. J Bio Sci 20:99–108
Janeiro LV, Ballester A, Vieitez AM (1997) In vitro response of encapsulated somatic embryos of camellia. Plant Cell Tiss Organ Cult 51:119–125
Kamada H, Kobayashi K, Kiyosue T, Harada H (1989) Stress induced somatic embryogenesis in carrot and its application to synthetic seed production. In Vitro Cell Dev Biol 25:1163–1166
Kavyashree R, Gayatri MC, Revanasiddaiah HM (2006) Propagation of mulberry variety-S54 by synseeds of axillary bud. Plant Cell Tiss Organ Cult 84:245–249
Khan MK, Sharma T, Misra P, Shukla PK, Singh Y, Ramteke PW (2013) Production of plantlets on different substrates from encapsulated in vitro nodal explants of *Stevia rebaudiana*. Int J Recent Sci Res 4:211–215
Khor E, Loh CS (2005) Artificial seeds. In: Nedovic V, Willaert R (eds) Applications of cell immobilization biotechnology. Springer, Berlin, pp 527–537
Kikowska M, Thiem B (2011) Alginate-encapsulated shoot tips and nodal segments in micropropagation of medicinal plants. A review. Herb Pol 57:45–57
Kim MA, Park JK (2002) High frequency plant regeneration of garlic (*Allium sativum* L.) calli immobilized in calcium alginate gel. Biotechnol Bioproc Eng 7:206–211
Kitto SL, Janick J (1982) Polyox as an artificial seed coat for a sexual embryos. Hortic Sci 17:448
Kumar V, Chandra S (2014) High frequency somatic embryogenesis and synthetic seed production of the endangered species *Swertia chirayita*. Biologia 69(2):186–192
Kumar GK, Thomas TD (2012) High frequency somatic embryogenesis and synthetic seed production in *Clitoria ternatea* Linn. Plant Cell Tiss Organ Cult 110:141–151
Kumar S, Rai MK, Singh N, Mangal M (2010) Alginate-encapsulation of shoot tips of jojoba [*Simmondsia chinensis* (link)

Schneider] for germplasm exchange and distribution. Physiol Mol Biol Plants 16:379–382
Kumara Swamy M, Balasubramanya S, Anuradha M (2009) Germplasm conservation of patchouli (*Pogostemon cablin* Benth.) by encapsulation of in vitro derived nodal segments. Int J Biodivers Conserv 1:224–230
Lambardi M, Benelli C, Ozudogru EA, Ozden-Tokatli Y (2006) Synthetic seed technology in ornamental plants. Floricult Ornam Plant Biotechnol 2:347–354
Lata H, Chandra S, Khan IA, Elsohly MA (2009) Propagation through alginate encapsulation of axillary buds of *Cannabis sativa* L.—an important medicinal plant. Physiol Mol Biol Plants 15:79–86
Lata H, Chandra S, Mehmedic Z, Khan IA, El-Sohly MA (2012) In vitro germplasm conservation of high D9-tetrahydrocannabinol yielding elite clones of *Cannabis sativa* L. under slow growth conditions. Acta Physiol Plant 34:743–750
Malabadi RV, van Staden J (2005) Storability and germination of sodium alginate encapsulated somatic embryos derived from the vegetative shoot apices of mature *Pinus patula* trees. Plant Cell Tiss Organ Cult 82:259–265
Mandal J, Pattnaik S, Chand PK (2000) Alginate encapsulation of axillary buds of *Ocimum americanum* L. (Hoary basil), *O. basilicum* (sweet basil), *O. gratissium* (shrubby basil) and *O. sanctum* (sacred basil). In Vitro Cell Dev Biol-Plant 36:287–292
Manjkhola S, Dhar U, Joshi M (2005) Organogenesis, embryogenesis and synthetic seed production in *Arnebia euchorma*—a critically endangered medicinal plant of the Himalaya. In Vitro Cell Dev Biol-Plant 41:244–248
Maqsood M, Mujib A, Siddiqui ZH (2012) Synthetic seed development and conservation to plantlet in *Catharanthus roseus* (L.). G Don. Biotechnology 11:37–43
Merkle SA, Parrott WA, Williams EG (1990) Applications of somatic embryogenesis and embryo cloning. Plant tissue culture: applications and limitations. Elsevier Science Publishers, New York, pp 67–101
Micheli M, Hafiz IA, Standardi A (2007) Encapsulation of in vitro-derived explants of olive (*Olea europaea* L. cv. Moraiolo) II. Effects of storage on capsule and derived shoots performance. Sci Hortic 113:286–292
Mishra J, Singh M, Palni LMS, Nandi SK (2011) Assessment of genetic fidelity of encapsulated microshoots of *Picrorhiza kurrooa*. Plant Cell Tiss Organ Cult 104:181–186
Mohanty P, Nongkling P, Das MC, Kumaria S, Tandon P (2013) Short-term storage of alginate-encapsulated protocorm-like bodies of *Dendrobium nobile* Lindl.: an endangered medicinal orchid from north-east India. 3 Biotech 3:235–239
Murashige T (1977) Plant cell and organ cultures as horticultural practices. Acta Hortic 78:17
Murthy KSR, Reddy MC, Kondamudi R (2013) Synthetic seeds - A novel approach for the conservation of endangered *C. spiralis* wt. and *C. pusilla*. Bangladesh J Sci Ind Res 48:39–42
Nagesh KS, Shanthamma C, Bhagyalakshmi N (2009) Role of polarity in de novo shoot bud initiation from stem disc explants of *Curculigo orchioides* Gaertn. and its encapsulation and storability. Acta Physiol Plant 31:699–704
Naik SK, Chand PK (2006) Nutrient-alginate encapsulation of in vitro nodal segments of pomegranate (*Punica granatum* L.) for germplasm distribution and exchange. Sci Hortic 108:247–252
Nirala NK, Das DK, Reddy MK, Srivastava PS, Sopory SK, Upadhyaya KC (2010) Encapsulated somatic embryos of grape (*Vitis vinifera* L.): an efficient way for storage, transport and multiplication of pathogen free plant material. Asia Pac J Mol Biol Biotechnol 18:159–162
Nishitha IK, Martin KP, Ligimol Beegum AS, Madhusoodanan PV (2006) Microproapagation and encapsulation of medicinally important *Chonemorpha grandiflora*. In Vitro Cell Dev Biol-Plant 42:385–388
Norstog K (1979) Embryo culture as a tool in the study of comparative and developmental morphology. In: Sharp WR, Larsen PO, Paddock EF, Raghavan V (eds) Plant cell and tissue culture: principles and applications. Ohio State University Press, Columbus, pp 179–202
Nower AB (2014) In vitro propagation and synthetic seeds production: an efficient method for *Stevia rebaudiana* Bertoni. Sugar Tech 16:100–108
Parveen S, Shahzad A (2014) Encapsulation of nodal segments of *Cassia angustifolia* Vahl. for short-term storage and germplasm exchange. Acta Physiol Plant 36:635–640
Pattnaik SK, Chand PK (2000) Morphogenic response of the alginate-encapsulated axillary buds from in vitro shoot cultures of six mulberries. Plant Cell Tiss Organ Cult 60:177–185
Phunchindawan M, Hirata K, Sakai A, Miyamoto K (1997) Cryopreservation of encapsulated shoot primordia induced in horseradish (*Armoracia rusticana*) hairy root cultures. Plant Cell Rep 16:469–473
Piątczak E, Wysokińska H (2013) Encapsulation of *Centaurium erythraea* Rafn—an efficient method for regeneration of transgenic plants. Acta Biol Crac Ser Bot 55(2):37–44
Piccioni E, Standardi A (1995) Encapsulation of micropropagated buds of six woody species. Plant Cell Tiss Organ Cult 42:221–226
Pinker I, Abdel-Rahman SSA (2005) Artificial seed for propagation of *Dendranthema* × *grandiflora* (Ramat.). Propag Ornam Plants 5:186–191
Pond S, Cameron H (2003) Artificial seeds. In: Thomas B, Murphy DJ, Murray BG (eds) Encyclopedia of applied plant sciences, vol 3. Elsevier, Oxford, pp 1379–1388
Prasad A, Singh M, Yadav NP, Mathura AK, Mathura A (2014) Molecular, chemical and biological stability of plants derived from artificial seeds of *Centella asiatica* (L.) Urban—an industrially important medicinal herb. Ind Crops Prod 60:205–211
Prewein C, Wilhelm E (2003) Plant regeneration from encapsulated somatic embryos of pedunculate oak (*Quercus robur* L.). In Vitro Cell Dev Biol-Plant 39:613–617
Rai MK, Jaiswal VS, Jaiswal U (2008) Encapsulation of shoot tips of guava (*Psidium guajava* L.) for short-term storage and germplasm exchange. Sci Hortic 118:33–38
Rai MK, Asthana P, Singh SK, Jaiswal VS, Jaiswal U (2009) The encapsulation technology in fruit plants—a review. Biotechnol Adv 27:671–679
Rawat JM, Rawat B, Mehrotra S (2013) Plant regeneration, genetic fidelity, and active ingredient content of encapsulated hairy roots of *Picrorhiza kurrooa* Royle ex Benth. Biotechnol Lett 35:961–968
Ray A, Bhattacharya S (2010) Storage and conversion of *Eclipta alba* synseeds and RAPD analysis of the converted plantlets. Biol Plant 54:547–550
Redenbaugh K (1990) Application of artificial seeds to tropical crops. Hort Science 25:251–255
Redenbaugh K, Nichol J, Kossler ME, Paasch BD (1984) Encapsulation of somatic embryos for artificial seed production. In Vitro Cell Dev Biol-Plant 20:256–257
Redenbaugh K, Fujii JA, Slade D (1991) 'Synthetic seed technology' in scale-up and automation in plant propagation. In: Vasil IK (ed) Cell culture and somatic cell genetics of plants. Academic Press, New York, pp 35–74
Redenbaugh K, Fujii JA, Slade D (1993) Hydrated coatings formsynthetic seeds. In: Redenbaugh K (ed) Synseeds: application of synthetic seeds to crop improvement. CRC Press, Boca Raton, pp 305–327

Remya M, Bai VNV, Mutharaian VN (2013) In vitro regeneration of *Aristolochia tagala* and production of artificial seeds. Biol Plant 57:210–218

Rizkalla AA, Elden AMB, Ottai MES, Nasr MI, Esmail MNM (2012) Development of artificial seed technology and preservation in sugar beet. Sugar Tech 14:312–320

Saha S, Sengupta C, Ghosh P (2014a) Molecular and phytochemical analyses to assess genetic stability in alginate-encapsulated microshoots of *Ocimum gratissimum* L. following in vitro storage. Nucleus 57:33–43

Saha S, Sengupta C, Ghosh P (2014b) Encapsulation, short-term storage, conservation and molecular analysis to assess genetic stability in alginate-encapsulated microshoots of *Ocimum kilimandscharicum* Guerke. Plant Cell Tiss Organ Cult doi:10.1007/s11240-014-0618-x

Saiprasad GVS (2001) Artificial seeds and their applications. Resonance 5:39–47

Saiprasad GVS, Polisetty R (2003) Propagation of three orchid genera using encapsulated protocorm-like bodies. In Vitro Cell Dev Biol-Plant 39:42–48

Sakhanokho HF, Pounders CT, Blythe EK (2013) Alginate encapsulation of *Begonia* microshoots for short-term storage and distribution. Sci World J 2013:1–7

Senaratna T (1992) Artificial seeds. Biotechnol Adv 10:379–392

Shaheen A, Shahzad A (2015) Nutrient encapsulation of nodal segments of an endangered white cedar for studies of regrowth, short term conservation and ethylene inhibitors influenced ex vitro rooting. Ind Crops Prod 69:204–211

Sharma S, Shahzad A (2012) Encapsulation technology for short-term storage and conservation of a woody climber, *Decalepis hamiltonii* Wight and Arn. Plant Cell Tiss Organ Cult 111:191–198

Sharma S, Shahzad A, Jan N, Sahai A (2009a) In vitro studies on shoot regeneration through various explants and alginate-encapsulated nodal segments of *Spilanthes mauritiana* DC., an endangered medicinal herb. Int J Plant Dev Biol 3:56–61

Sharma S, Shahzad A, Sahai A (2009b) Artificial seeds for propagation and preservation of *Spilanthes acmella* (L.) Murr., a threatened pesticidal plant species. Int J Plant Dev Biol 3:62–64

Sharma S, Shahzad A, Teixeira da Silva JA (2013) Synseed technology—a complete synthesis. Biotechnol Adv 31:186–207

Sharma S, Shahzad A, Kumar J, Anis M (2014) In vitro propagation and synseed production of scarlet salvia (*Salvia splendens*). Rend Fis Acc Lincei 25:359–368

Siddique I, Anis M (2009) Morphogenic response of the alginate encapsulated nodal segments and antioxidative enzymes analysis during acclimatization of *Ocimum basilicum* L. J Crop Sci Biotechnol 12:233–238

Singh KK (2008) In vitro plant regeneration of an endangered Sikkim Himalayan rhododendron (*R. maddeni* Hook. F.) from alginate-encapsulated shoot tips. Biotechnol 7:144–148

Singh AK, Chand S (2010) Plant regeneration from alginate-encapsulated somatic embryos of *Dalbergia sissoo* Roxb. Indian J Biotechnol 9:319–324

Singh AK, Sharma M, Varshney R, Agarwal SS, Bansal KC (2006a) Plant regeneration from alginate to encapsulated shoot tips of *Phyllanthus amarus* Schum and Thonn, a medicinally important plant species. In Vitro Cell Dev Biol-Plant 42:109–113

Singh AK, Varshney R, Sharma M, Agarwal SS, Bansal KC (2006b) Regeneration of plants from alginate-encapsulated shoot tips of *Withania somnifera* (L.) Dunal, a medicinally important plant species. J Plant Physiol 163:220–223

Singh B, Sharma S, Rani G, Virk GS, Zaidi AA, Nagpal A (2007) In vitro response of encapsulated and non-encapsulated somatic embryos of *Kinnow mandarin* (*Citrus nobilis Lour* × *C. deliciosa Tenora*). Plant Biotechnol Rep 1:101–107

Singh SK, Rai MK, Asthana P, Pandey S, Jaiswal VS, Jaiswal U (2009) Plant regeneration from alginate-encapsulated shoot tips of *Spilanthes acmella* (L.) Murr., a medicinally important and herbal pesticidal plant species. Acta Physiol Plant 31:649–653

Singh SK, Rai MK, Asthana P, Sahoo L (2010) Alginate-encapsulation of nodal segments for propagation, short-term conservation and germplasm exchange and distribution of *Eclipta alba* (L.). Acta Physiol Plant 32:607–610

Srivastava V, Khan SA, Banerjee S (2009) An evaluation of genetic fidelity of encapsulated microshoots of the medicinal plant: *Cineraria maritima* following 6 months of storage. Plant Cell Tiss Organ Cult 99:193–198

Standardi A, Piccioni E (1998) Recent perspectives on synthetic seed technology using nonembryogenic in vitro-derived explants. Int J Plant Sci 159:968–978

Subhashini Devi P, Satyanarayana B, Arundhati A, Raghava Rao T (2014) Plant regeneration from encapsulated nodal segments of *Sterculia urens* Roxb., an endangered gum-yielding tree. J Crop Sci Biotech 17:21–25

Sudarshana MS, Rajashekar N, Niranjan MH, Borzabad RK (2013) In vitro regeneration of multiple shoots from encapsulated somatic embryos of *Artemisia vulgaris,* L. IOSR J Pharma Biol Sci 6:11–15

Sundararaj SG, Agrawal A, Tyagi RK (2010) Encapsulation for in vitro short-term storage and exchange of ginger (*Zingiber officinale* Rosc.) germplasm. Sci Hortic 125:761–766

Swamy MK, Balasubramanya S, Anuradha M (2009) Germplasm conservation of patchouli (*Pogostemon cablin* Benth.) by encapsulation of in vitro derived nodal segments. Int J Biol Control 1:224–230

Swaroopa G, Nigam N, Maniyam A (2007) Germplasm conservation of selected lines of *Coleus forskohlii* (Willd.) Briq by nodal segment encapsulation. Phytomorphol 57:221–225

Tabassum B, Nasir IA, Farooq AM, Rehman Z, Latif Z, Husain T (2010) Viability assessment of in vitro produced synthetic seeds of cucumber. Afr J Biotechnol 9:7026–7032

Tsvetkov I, Jouve L, Hausman JF (2006) Effect of alginate matrix composition on re-growth of in vitro-derived encapsulated apical microcuttings of hybrid aspen. Biol Plant 50:722–724

Upadhyay R, Kashyap SP, Singh CS, Tiwari KN, Singh K, Singh M (2014) Ex situ conservation of *Phyllanthus fraternus* Webster and evaluation of genetic fidelity in regenerates using dna-based molecular marker. Appl Biochem Biotechnol. doi:10.1007/s12010-014-1175-9

Varshney A, Anis M (2014) Synseed conception for short-term storage, germplasm exchange and potentialities of regeneration genetically stable plantlets of desert date tree (*Balanites aegyptiaca* Del.). Agrofor Syst 88:321–329

Williams EG, Maheswaran G (1986) Somatic embryogenesis: factors influencing coordinated behaviour of cells as an embryogenic group. Ann Bot 57:443–462

Zych M, Furmanowa M, Krajewska-Patan A, Łowicka A, Dreger M, Mendlewska S (2005) Micropropagation of *Rhodiola kirilowii* plants using encapsulated axillary buds and callus. Acta Biol Crac Ser Bot 47:83–87