Mohammad Faisal
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Synthetic Seeds

Germplasm Regeneration, Preservation and Prospects

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ISBN 978-3-030-24630-3 ISBN 978-3-030-24631-0 (eBook)
https://doi.org/10.1007/978-3-030-24631-0

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

The book is dedicated to my supervisors

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Preface

Synthetic or artificial seeds are described as alginate-encapsulated somatic embryos, vegetative buds, or any other micropropagules that can be used as seeds and converted into plantlets after propagating in vitro or in vivo conditions and also sustain the regeneration potential after low temperature storage. Production of synthetic or artificial seeds using micropropagules opens up new vistas in agricultural biotechnology that helped to overcome the challenges that face important economic and medicinal plant species. Encapsulated propagules could be used for in vitro regeneration and mass multiplication at reasonable cost. In addition, these propagules may be use for germplasm preservation of elite plant species and exchange of plant materials between national and international laboratories. Besides, the technology has been successfully utilizing for cryopreservation via encapsulation-dehydration, and encapsulation-vitrification for germplasm storage of elite plant species. Synthetic seeds are reasonably inexpensive to produce and easy to handle, plant, and transport and have great advantages in comparison with traditional in vitro culture methods. The aim of this book is to provide relevant state-of-the-art findings on methods, application, and prospects of synthetic or artificial seeds. Being involved in this area, we comprehend that information on encapsulation and synseed production is still obscure, and there is no single book available on this aspect.

The intended volume comprised several chapters on relevant topics contributed by experts working in the field of plant biotechnology so as to make available a comprehensive treatise designed to provide an in-depth analysis of the subject in question. The book is a compilation of 22 chapters having relevant text, tables, and illustration describing the experimental work on encapsulation and synthetic seeds production, for regeneration, multiplication, germplasm preservation, exchange, and crop improvement in several plant species which will be useful in planning and execution of various experiments smoothly and effectively.

The present book aimed to induce new outlooks to scientists/researcher who are unfamiliar with synthetic seeds and will be very helpful in various present and future researches in different areas of plant biotechnology, cryobiology, molecular biology, plant physiology, and seed biology.

We are extremely thankful to all the contributors who wholeheartedly welcomed our invitation and agreed to contribute chapters to embellish information on synthetic seed production, thus helped in this endeavor.

Riyadh, Saudi Arabia
May 19, 2019

Mohammad Faisal
Abdulrahman A. Alatar

Acknowledgments

We the Editors are thankful and acknowledge the generous support established from all the contributors who spared precious time in scripting the chapters for this edited volume and also genuinely thank the publisher for their cooperation in making this book a reality. We are grateful to the reviewers for their thoughtful comments and suggestions, which have been very helpful for improving the manuscripts. We express our heartfelt thanks to Mr. Eslam M. Abdel-Salam and Ahmad A. Qahtan for their spontaneous help, suggestions, and constant encouragement throughout the work. We express our special thanks to the King Saud University for all support.

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Mohammad Faisal has over 12 years of research experience in Plant Biotechnology currently working as Associate Professor in the Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia. He has been the recipient of Plant Biotechnologist Award 2017 by SESR, India, and Scientist of the Year Award 2015 by NESA and several National and International Fellowships. He holds membership of several academic bodies/societies and Fellow of the SESR. He has published over 82 research articles, 5 book chapters, and 4 books. He is in the editorial board of the several reputed journals.

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An Introduction to Synthetic Seeds: Production, Techniques, and Applications

Ahmad A. Qahtan, Eslam M. Abdel-Salam, Abdulrahman A. Alatar, Qiao-Chun Wang, and Mohammad Faisal

Abstract Recent breakthroughs in in vitro culturing of plant cell tissue have helped to overcome the challenges that face important economic and medicinal plant species. Micropropagation and encapsulation techniques have been combined to develop a new tool, known as "synseed," which has the advantages of both technologies. Synseeds or artificial seeds are alginate encapsulated somatic embryos, vegetative buds, or any other micropropagules that can be used as seeds and germinated into plantlets after propagating under in vitro or in vivo conditions and that can also sustain the regeneration potential after low temperature storage. Encapsulated propagules may be used for germplasm preservation of elite plant species and exchange of plant materials between national and international laboratories. In addition, the technology has been successfully utilized for cryopreservation via encapsulation-dehydration, and encapsulation–vitrification for the germplasm storage of elite plant species. In this paper, we provide updated and comprehensive information on synseed technology, with a particular focus on the importance of explant selection for successful synseed production and on the matrices used as an encapsulation material for synseeds. Furthermore, the limiting factors that hinder the progress of synseed technology and related future perspectives are also discussed.

Keywords Conservation · Elite species · Germplasm · Synseeds · Tissue culture

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M. Faisal, A. A. Alatar (eds.), *Synthetic Seeds*,
https://doi.org/10.1007/978-3-030-24631-0_1

1 Introduction

Synthetic seeds, or artificial seeds, are encapsulated plant tissues such as shoot buds, axillary buds, somatic embryos, shoot tips, cell aggregates, or any other tissues that can be cultured as a seed and grown into a complete plant under either in vitro or in ex vitro conditions and have the potential to retain their viability after cold storage (Magray et al. 2017; Rihan et al. 2017). Previously, artificial seeds were produced by encapsulation of the somatic embryo; however, in recent years, synseeds have been produced by the encapsulation of various in vitro-derived propagules such as nodal segments containing axillary buds, apical shoot buds, and stem segments (Bapat et al. 1987; Danso and Ford-Lloyd 2003; Rai et al. 2008a). Murashige (1977) was the first researcher to discuss the concept of artificial seeds, while desiccated artificial carrot seeds were first produced by Kitto and Janick (1982). Later, Redenbaugh et al. (1984) successfully developed a method for synseed production by encapsulation of somatic embryos of alfalfa in sodium alginate. Similarly, Bapat et al. (1987) also succeeded in producing synthetic seeds of *Morus indica* from shoot buds encapsulated in alginate and in agar as an alternative to somatic embryos.

During recent decades, there has been great interest in the use of synseed technology to produce artificial seeds, especially for the plants that have low seed viability, seedless fruit, and poor germination rates, as well as for plants that depend on mycorrhizal–fungal symbiosis for germination (Rai et al. 2008b; Gantait et al. 2015). Furthermore, synseed technology may be useful in the selection of genotypes and sterile unsteady genotypes; germplasm preservation of elite planting materials; and in vitro propagation of endangered, rare, and commercially important plants (Danso and Ford-Lloyd 2003; Naik and Chand 2006; Gantait et al. 2015). Additionally, encapsulation technology provides easy handling, short- and long-term storage capacity, genetic uniformity, and low cost quality plant materials; it also allows for transportation and exchange of germplasm between national and international laboratories (Rai et al. 2009; Parveen and Shahzad 2014). Synseed technology has been successfully applied to numerous plant species, including medicinal plants, ornamentals, vegetables, fruits, cereals, and forest trees (Table 1). Schematic representation of synseed production is depicted in Fig. 1.

2 Selection of Plant Materials

Selection of the most appropriate explant as a starting material is a key factor for the successful production of synseeds. Synseeds have been produced from different plant propagules, which are discussed in this section.

Table 1 Application of synseed technology in different plant species

Plant species	Explant	Concentration of sodium alginate	Concentration of calcium chloride	References
Allium sativum	Callus	1.5%	50 mM	Kim and Park (2002)
Manihot esculenta	Nodal cuttings and shoot tips	3%	100 mM	Danso and Ford-Lloyd (2003)
Paulownia elongata	Somatic embryos	1, 2.5, and 3%	50, 60, 80 mM	Ipekci and Gozukirmizi (2003)
Oryza sativa	Somatic embryos	4%	1.5%	Kumar et al. (2005)
Rotula aquatica	Somatic embryos	3%	50 mM	Chithra et al. (2005)
Pinus patula	Somatic embryos	1.5, 2, 2.5, and 3%	100 mM	Malabadi and Staden (2005)
Rhodiola kirilowii	Axillary buds and callus	4 and 5%	50 mM	Zych et al. (2005)
Arnebia euchroma	Somatic embryos	3%	100 mM	Manjkhola et al. (2005)
Hibiscus moscheutos	Nodal segments	2.75%	50 mM	West et al. (2006)
Punica granatum	Nodal segments	1–6%	50, 75, 100, and 125 mM	Naik and Chand (2006)
Chonemorpha grandiflora	Shoot tips	3%	50 mM	Nishitha et al. (2006)
Tylophora indica	Nodal segments	1–5%	25, 50, 75, 100, and 200 mM	Faisal and Anis (2007)
	Nodal segments	2–5%	75 and 100 mM	Gantait et al. (2017b)
Pogonatherum paniceum	Shoot buds	3%	2%	Wang et al. (2007)
Pinus radiata	Somatic embryos	1, 2, and 3%	50, 75, and 100 mM	Aquea et al. (2008)
Psidium guajava	Shoot tips	2–4%	100 mM	Rai et al. (2008b)
Nothofagus alpina	Somatic embryos	2, 3, and 4	5.5, 14, and 15 g/L^{-1}	Cartes et al. (2009)
Zingiber officinale	Microshoots	4%	100 mM	Sundararaj et al. (2010)
Vitex negundo	Nodal segments	2–5%	25, 50, 75, 100, and 200 mM	Ahmad and Anis (2010)
Eclipta alba	Nodal segments	2–5%	50, 100, and 150 mM	Singh et al. (2010)
Solanum nigrum	Shoot tip	2–4%	100 mM	Verma et al. (2010)

(continued)

Table 1 (continued)

Plant species	Explant	Concentration of sodium alginate	Concentration of calcium chloride	References
Khaya senegalensis	Shoot tips	3%	100 mM	Hung and Trueman (2011)
Salvia officinalis	Shoot tips	2 and 3%	50 mM	Grzegorczyk and Wysokińska (2011)
Corymbia torelliana × *C. citriodora*	Shoot tips and nodal segments	3%	100 mM	Hung and Trueman (2012)
Ruta graveolens	Nodal segments	2–5%	25, 50, 75, 100, and 200 mM	Ahmad et al. (2012)
Rauvolfia tetraphylla	Microshoots	1–5%	25, 50, 75, 100, and 200 mM	Alatar and Faisal (2012)
Clitoria ternatea	Somatic embryos	3, 4, and 5%	75 and 100 mM	Kumar and Thomas (2012)
Rauvolfia serpentina	Nodal segments	3%	100 mM	Faisal et al. (2012)
	Shoot tips	1–5%	75 and 100 mM	Gantait et al. (2017a)
Cymbidium	Protocorm-like bodies	3, 3.5, and 4%	100 mM	da Silva (2012)
Dendrobium nobile	Protocorm-like bodies	3%	100 mM	Mohanty et al. (2013)
Rhinacanthus nasutus	Somatic embryos	4%	100 mM	Cheruvathur et al. (2013)
Withania somnifera	Nodal segments with axillary buds	2–5%	25, 50, 75, 100, and 200 mM	Fatima et al. (2013)
Aristolochia tagala	Microshoots	2, 3, and 4%	68 mM	Remya et al. (2013)
Ceropegia bulbosa	Nodal explants	1–5%	100 mM	Dhir and Shekhawat (2013)
Phyllanthus fraternus	Nodal segments	1, 1.5, 2, 2.5, 3, and 4%	25, 50, 75, 100, and 200 mM	Upadhyay et al. (2014)
Ocimum gratissimum	Microshoots	1–5%	25, 50, 75, 100, and 150 mM	Saha et al. (2014)
Terminalia arjuna	Shoot tips	2–5%	100 mM	Gupta et al. (2014)
Cucumis sativus	Shoot tips	1–5%	25, 50, 75, and 100 mM	Adhikari et al. (2014)

(continued)

Table 1 (continued)

Plant species	Explant	Concentration of sodium alginate	Concentration of calcium chloride	References
Anethum graveolens	Somatic embryos	1–5%	75 and 100 mM	Dhir et al. (2014)
Balanites aegyptiaca	Nodal segments	2–5%	25, 50, 75, 100, and 200 mM	Varshney and Anis (2014)
Sterculia urens	Nodal segments	2, 4, and 6	100 mM	Devi et al. (2014)
Cassia angustifolia	Nodal segments	1–5%	25, 50, 75, 100, and 200 mM	Parveen and Shahzad (2014)
Mondia whitei	Somatic embryos	1–4%	75, 100, and 125 mM	Baskaran et al. (2015)
Vitex trifolia	Nodal segments	1–5%	25, 50, 75, 100, and 200 mM	Ahmed et al. (2015)
	Nodal segments	2–5%	25, 50, 75, 100, and 200 mM	Alatar et al. (2017)
Gossypium hirsutum	Axillary buds	1–5%	25, 50, 75, 100, and 200 mM	Hu et al. (2015)
Ledebouria revoluta	Somatic embryos	1.5, 3, and 4.5%	150 mM Ca $(NO_3)_2$	Haque and Ghosh (2016)
Solanum tuberosum	Axillary buds	2.5, 3, and 3.5%	1 and 1.5%	Ghanbarali et al. (2016)
Curcuma amada	Somatic embryos	1–4%	100 mM	Raju et al. (2016)
Erythrina variegata	Nodal segments	1–4%	25, 50, 75, 100, and 200 mM	Javed et al. (2017)
Urginea altissima	Shoot tips	3%	100 mM	Baskaran et al. (2017)
Spathoglottis plicata	Protocorm-like bodies	1.5, 3, and 4.5%	3% calcium nitrate	Haque and Ghosh (2017)
Capparis decidua	Nodal segments	2–5%	25, 50, 75, 100, and 125 mM	Siddique and Bukhari (2018)
Ceropegia barnesii	Nodes	2, 3, and 4%	60 mM	Ananthan et al. (2018)
Rosa damascena trigintipetala	Axillary buds	2, 4, and 5%	75 and 100 mM	Attia et al. (2018)
Salix tetrasperma	Nodal segments	1–5%	25, 50, 75, 100, and 200 mM	Khan et al. (2018)
Plumbago rosea	Nodal axillary buds	2.5, 3, 4, and 5%	50, 75, 100, and 200 mM	Prakash et al. (2018)

(continued)

Table 1 (continued)

Plant species	Explant	Concentration of sodium alginate	Concentration of calcium chloride	References
Taraxacum pieninicum	Shoot tips	3%	100 mM	Kamińska et al. (2018)
Saccharum officinarum	Microshoots	2, 3, and 4%	25, 50, 75, 100, and 125 mM	Badr-Elden (2018)

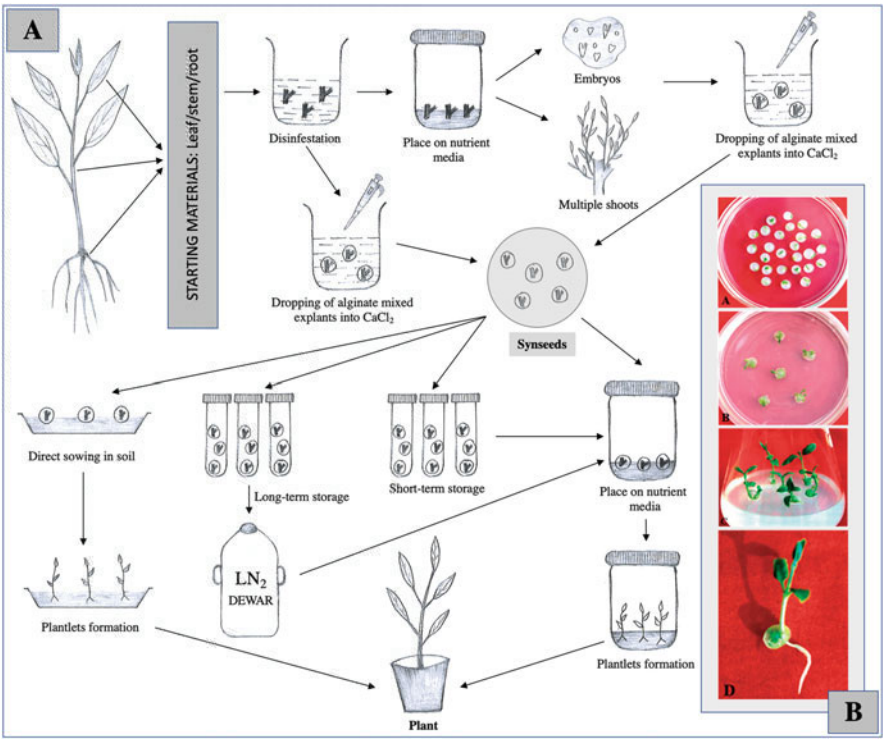

Fig. 1 (**a**) Schematic representation of synthetic seed production; (**b**) synseed seed produced from nodal segments of *Tylophora indica*. Source: Faisal and Anis (2007)

2.1 Somatic Embryo

Bipolar structures that contain both the shoot and root poles are described as somatic embryos. These are the most suitable material for synseed seed production because of their polar nature, which means they are able to develop roots and shoots in a single step (Standardi and Piccioni 1998; Sharma et al. 2013). Somatic embryos have been successfully used for synseed production in several plant species including

Rotula aquatica (Chithra et al. 2005), *Oryza sativa* (Kumar et al. 2005), *Pinus radiata* (Aquea et al. 2008), *Nothofagus alpina* (Cartes et al. 2009), *Dalbergia sissoo* (Singh and Chand 2010), *Clitoria ternatea* (Kumar and Thomas 2012), *Rhinacanthus nasutus*, *Hemidesmus indicus* (Cheruvathur et al. 2013), *Anethum graveolens* (Dhir et al. 2014), *Mondia whitei* (Baskaran et al. 2015), *Ledebouria revoluta* (Haque and Ghosh 2016), and *Curcuma amada* (Raju et al. 2016). However, the deficient and asynchronous maturation of the embryonic pole is the basic problem for synseed production in woody species (Cartes et al. 2009; da Silva and Malabadi 2012). To address this problem, several researchers proposed using compounds such as nutrients, growth regulators, herbicides, anti-pathogens, bio-fertilizers, and bio-controllers (Kumar et al. 2005; Cartes et al. 2009). Somatic embryos of *Pinus patula* that had been encapsulated with 2.5% sodium alginate and dissolved in DCR basal medium had a germination rate of 89% (Malabadi and Staden 2005). Cheruvathur et al. (2013) reported that synseeds produced from somatic embryos had a 100% regeneration rate in MS with 2 μM kinetin and 0.5 μM IBA.

2.2 Nodal Segment and Shoot Tips

Nodal segments with axillary bud (microcuttings) are the most common propagules used for synseed production. This is probably due to the relative ease with which these explants are produced once the micropropagation system has been established and because they have the ability to retain viability in terms of sprouting and conversion potential even after a considerable period of storage, which is required for germplasm exchange (Piccioni and Standardi 1995; Ahmad et al. 2012). Nodal segments have been frequently used for synthetic seeds in several plants, such as *Tylophora indica* (Faisal and Anis 2007; Gantait et al. 2017b), *Eclipta alba* (Singh et al. 2010), *Vitex negundo* (Ahmad and Anis 2010), *Ruta graveolens* (Ahmad et al. 2012), *Rauvolfia serpentina* (Faisal et al. 2012), *Cannabis sativa* (Lata et al. 2012), *Ceropegia bulbosa* (Dhir and Shekhawat 2013), *Sterculia urens* (Devi et al. 2014), *Balanites aegyptiaca* (Varshney and Anis 2014), *Phyllanthus fraternus* (Upadhyay et al. 2014), *Centella asiatica* (Prasad et al. 2014), *Vitex trifolia* (Ahmed et al. 2015; Alatar et al. 2017), *Gossypium hirsutum* (Hu et al. 2015), *Solanum tuberosum* (Ghanbarali et al. 2016), *Erythrina variegata* (Javed et al. 2017), *Capparis decidua* (Siddique and Bukhari 2018), *Salix tetrasperma* (Khan et al. 2018), and *Rosa* × *damascena* f. *trigintipetala* (Attia et al. 2018).

Apical meristems or shoot tips were also used for encapsulation of explants in several plant species, such as *Psidium guajava* (Rai et al. 2008b), *Solanum nigrum* (Verma et al. 2010), *Khaya senegalensis* (Hung and Trueman 2011), *Salvia officinalis* (Grzegorczyk and Wysokińska 2011), *Cucumis sativus* (Adhikari et al. 2014), *Terminalia arjuna* (Gupta et al. 2014), *Rauvolfia serpentina* (Gantait et al. 2017a), *Urginea altissima* (Baskaran et al. 2017), and *Taraxacum pieninicum* (Kamińska et al. 2018). Hung and Trueman (2012) successfully developed methods for synseed production in *Corymbia torelliana* × *C. citriodora* using nodal segments and shoot tips. They found higher regrowth abilities, with about 76–100% regrowth

from nodal segments and 78–100% from encapsulated shoot tips, using full and half-strength MS media, respectively.

2.3 Callus and Protocorm-Like Bodies

Generally, calluses are not often used in production of synseeds. This could be attributed to the undifferentiated nature of calluses, which have several requirements for successful differentiation that limits the utility and acceptability of the use of calluses in the production of synseeds (Gantait et al. 2015). There have been very few successful attempts to produce synseeds by encapsulating calluses. In a previous study, calluses of *Allium sativum* obtained in vitro from shoot tip explants were encapsulated using calcium chloride and sodium alginate and regenerated on semi-solid ½MS medium without growth regulators, and achieved a regeneration frequency of 95% (Kim and Park 2002). Similarly, Zych et al. (2005) successfully encapsulated the differentiating calluses derived from the hypocotyls of *Rhodiola kirilowii* plants. The encapsulated calluses can be stored at a low temperature (4 °C) for 6 weeks and exhibited regeneration potential after transfer without hormones, with 95% regeneration frequency.

The production of synthetic seeds using protocorm-like bodies (PLBs) is mainly used for orchids because they produce tiny, non-endospermic seeds. Several studies have investigated the feasibility of encapsulating PLBs and culturing the produced seeds directly in the soil, without in vitro regeneration (e.g., Ara et al. 2000; Saiprasad 2001; Vij et al. 2001; Saiprasad and Polisetty 2003). Corrie and Tandon (1993) found that encapsulated PLB of *Cymbidium giganteum* could be cultivated directly in sterilized soil with a regeneration frequency of 88 and 64% on sand and on a sand and soil mixture, respectively. Seeking to optimize seed production in three orchid genera (*Dendrobium*, *Oncidium*, and *Cattleya*), Saiprasad and Polisetty (2003) tried different developmental stages of PLBs and various combinations of sodium alginate, $CaCl_2$, and MS salts. They successfully encapsulated fractionated PLBs after 13–15 days of culture using 3% sodium alginate and 75 mM $CaCl_2$. Seeds of *Dendrobium*, *Oncidium*, and *Cattleya* were stored at 4 °C for 75, 60, and 30 days, respectively, and had a regeneration potential of more than 88%. Sarmah et al. (2010) used PLB produced from 6-week-old leaves of *Vanda coerulea* plants for encapsulation using 100 mM $CaCl_2$ solution for 30 min, and the encapsulated PLBs were stored for 100 days at 4 °C.

3 Selection of the Encapsulation Matrix

The encapsulation material is considered to be a critical factor for the production of uniform synseeds. The encapsulation material should be consistent enough to allow seed handling without breakage, but weak enough to allow the bud to break free

from the capsule upon regrowth (Redenbaugh et al. 1986). This balance between synseed hardness and softness can be achieved by encapsulating explants with sodium alginate hydrogel (Rai et al. 2009; Gantait et al. 2015). Sodium alginate is the most commonly used substance for encapsulation of explants; however, there are other agents such as sodium alginate with gelatin, potassium alginate, sodium pectate, and carrageenan that are used for encapsulation. In general, sodium alginate has been shown to be the most commonly used for encapsulation because of its useful thickness, low cost, fast gelation, and nontoxic nature (Rai et al. 2009; Cheruvathur et al. 2013; Gantait et al. 2015; Rihan et al. 2017). It can also provide better protection for the covered explants against mechanical damage (Saiprasad 2001). The strength of encapsulated beads depends mainly on the concentration of sodium alginate and calcium chloride, as well as the mixing duration; however, it may vary for different explants and plant species (Rai et al. 2009; Rihan et al. 2017). Furthermore, the addition of nutrients and growth regulators to the encapsulation matrix is also an important factor for successful synseed production, as it increases the reliability of germination and the viability of the synseeds. These matrices are considered to be artificial endosperms, and they also play an important role in the storage of synseeds at low temperatures and in regrowth ability after transfer to germination media (Saiprasad 2001; Rihan et al. 2017).

In most studies, the optimum concentration for synthetic seed production has been reported to be 3% sodium alginate and 100 mM $CaCl_2$ for several plant species including *Manihot esculenta* (Danso and Ford-Lloyd 2003), *Tylophora indica* (Faisal and Anis 2007), *Psidium guajava* (Rai et al. 2008a, b), *Vitex negundo* (Ahmad and Anis 2010), *Eclipta alba* (Singh et al. 2010), *Solanum nigrum* (Verma et al. 2010), *Khaya senegalensis* (Hung and Trueman 2011), *Ruta graveolens* (Ahmad et al. 2012), *Rauvolfia tetraphylla* (Alatar and Faisal 2012), *Rauvolfia serpentina* (Faisal et al. 2012), *Dendrobium nobile* (Mohanty et al. 2013), *Ceropegia bulbosa* (Dhir and Shekhawat 2013), *Balanites aegyptiaca* (Varshney and Anis 2014), *Cucumis sativus* (Adhikari et al. 2014), *Vitex trifolia* (Ahmed et al. 2015; Alatar et al. 2017), *Mondia whitei* (Baskaran et al. 2015), *Curcuma amada* (Raju et al. 2016), *Erythrina variegata* (Javed et al. 2017), *Salix tetrasperma* (Khan et al. 2018), and *Taraxacum pieninicum* (Kamińska et al. 2018). Nevertheless, an encapsulation matrix of 2% sodium alginate with $CaCl_2$ of 50 mM was found to produce high quality beads in *Artemisia vulgaris* by encapsulating nodal segments (Sujatha and Kumari 2008). Wang et al. (2007) reported that *Pogonatherum paniceum* synseeds produced using 3% sodium alginate, 1% activated carbon, and 2% calcium chloride gave a higher conversion rate (61.58%) than synseeds prepared without activated carbon (44.06%). da Silva (2012) reported that 3.5% sodium alginate was the most appropriate concentration for the encapsulation of PLB in hybrid *Cymbidium*. Furthermore, 3% sodium alginate and 75 mM calcium chloride ($CaCl_2 \cdot 2H_2O$) were found to be the most appropriate combination for synseed production in *Tylophora indica* and *Rauvolfia serpentina* (Gantait et al. 2017a, b). Similarly, Siddique and Bukhari (2018) also found that 3% sodium alginate and 75 mM calcium chloride was the best suited matrix for synseed production in *Capparis decidua*.

4 Methods

4.1 Encapsulation Matrix

The required concentrations of sodium alginate solution (0.5–5.0% w/v) were prepared in liquid nutrient medium or double distilled water to form the gel matrix. Similarly, calcium chloride solutions were prepared at different concentrations (25–200 mM) in double distilled water to form the complexing agent. Both the gel matrix and complexing agent were autoclaved at 121 °C and 1.1 kg cm^{-2} pressure for 15 min.

4.2 Encapsulation

After preparation of gel matrix and complexing agent, selected plant materials (explants) were prepared for encapsulation as follows:

1. The propagules were dipped in 3% sodium alginate solution.
2. The mixture (propagules contained within sodium alginate) was placed into calcium chloride solution (100 mM) and left for 30–40 min to allow the alginate beads to harden, forming calcium alginate around the propagules.
3. Calcium alginate beads were washed with sterile double distilled water two to three times to remove traces of calcium chloride.
4. Synseeds were transferred to sterile filter paper and left for 5 min under the laminar air flow hood to dry.
5. The synseeds were then ready and could be stored at 4, 15, or 24 °C, depending on the intended use.

5 Applications of Synseeds

Synseeds have several applications in different fields of plant biotechnology and the conservation of rare or endangered plant species. These applications include in vitro or ex vitro (direct sowing) propagation of various plant species; short-, medium-, and long-term preservation of germplasm; and transportation and exchange of plant materials.

5.1 Propagation

Encapsulated explants are characterized by regrowth and conversion abilities after encapsulation and storage at low temperatures, when transferred to the germination

media (Micheli et al. 2007). Synseeds could be used for propagation and multiplication of rare and endangered plants, elite genotypes, seedless plants, medicinal plants, genetically engineered (modified) plants, and commercially important plants (Rai et al. 2009; Gantait et al. 2015). Synseeds can be efficiently cultivated in vitro, either on semi-solid culture medium or planting substrate (e.g., perlite, vermicompost, vermiculite, soil, soilrite, sand, or gravel) for conversion into complete plantlets (Sharma et al. 2013). Generally, the regrowth ability of explants encapsulated in calcium alginate beads into complete plantlets on nutrient-rich medium is more effective than on nutrient-deficient substrates (Mandal et al. 2000; Sharma et al. 2013). The concentration of plant growth regulators in the medium plays a crucial role in conversion and whole plant regeneration from encapsulated buds (Cheruvathur et al. 2013). The plant growth regulator requirement in nutrient medium significantly depends upon the plant species. Nishitha et al. (2006) reported that encapsulated shoot tips of *Chonemorpha grandiflora* had a 95% conversion to plantlets on medium containing 0.49 μM IBA and 11.7 μM silver nitrate. Plantlets showed a 90% survival after acclimatization in soil. Dhir and Shekhawat (2013) reported that maximum percentage response for conversion of synthetic seeds into plantlets in *Ceropegia bulbosa* was 100% on medium supplemented with 8.88 μM BA. Gupta et al. (2014) found that highest rate of plantlet conversion from encapsulated shoot tips in *Terminalia arjuna* on 0.14% gelrite-gelled MMS supplemented with 0.5 mg L^{-1} BAP and 0.1 mg L^{-1} NAA was 91.6%. Encapsulated somatic embryos of *Mondia whitei* had 95.7% survival and 73% germination rates (Baskaran et al. 2015). Baskaran et al. (2017) obtained 91% adventitious shoot regeneration from *Urginea altissima* encapsulated shoot tips on semi-solid MS medium containing 10 μM *m*T and 2 μM NAA. Siddique and Bukhari (2018) obtained the highest conversion rate of 93% from encapsulated nodal segment of *Capparis decidua*.

5.2 Short- and Medium-Term Conservation

Synseed technology offers strategies for the conservation of plant species through short- and medium-term preservation. These processes are generally known as slow-growth techniques. Appropriate storage conditions and a finite storage period are the most critical factors to maintain synseed viability during transportation and conservation, and these may lead to the successful commercialization of this technique (Sharma et al. 2013). The optimal storage temperature for short- or medium-term storage varies depending on the plant species. Generally, low temperature storage at 4 °C in a laboratory freezer has been found to be the most suitable conditions for synseeds of most plant species (Ray and Bhattacharya 2008; Parveen and Shahzad 2014; Ahmed et al. 2015; Alatar et al. 2017). The role of temperature on short- or medium-term storage of synseeds has been investigated by several researchers (Table 2). Faisal et al. (2013) reported that 4 °C was the optimal temperature for short-term storage (storage for up to 4 weeks), with a high conversion percentage (80.6%). In *Ceropegia bulbosa*, the conversion frequency of encapsulated nodal

Table 2 Short- and medium-term conservation of synthetic seeds

Plant species	Encapsulated explant	Storage conditions	Storage period	Regrowth rate (%)	Optimum	Post-storage treatment or culture	References
Pinus patula	Somatic embryo	2 °C 4 °C	0–120 days	73–89 61–73	73% at 2 °C after 120 days	½ DCR basal medium	Malabadi and Staden (2005)
Tylophora indica	Nodal segments	4 °C	0–8 weeks	50.3–91.3	72.3 after 4 weeks	MS + 2.5 µM BA + 0.5 µM NAA	Faisal and Anis (2007)
Rauvolfia serpentina	Shoot tips	4 °C	0–14 weeks	68.5–100		MS + 3% sucrose	Ray and Bhattacharya (2008)
	Nodal segments	4 °C	0–8 weeks	50–91.6	80% after 4 weeks	WPM + 5.0 µM BA + 1.0 µM NAA	Faisal et al. (2012)
Zingiber officinale	Microshoots	25 °C	2–12 weeks	13.0–86.7	53 after 8 weeks	MS + 2.5 mg/L BA	Sundararaj et al. (2010)
Vitex negundo	Nodal segments	4 °C	0–8 weeks	50–92.6		MS + 2.5 µM Kin + 1.0 µM NAA	Ahmad and Anis (2010)
Eclipta alba	Nodal segments	4 °C	0–60 days	51.2–100		MS + 0.88 µM BAP	Singh et al. (2010)
Decalepis hamiltonii	Nodal segments	4 °C	0–8 weeks	14–77	63.80 after 2 weeks	MS + 5 µM BA + 0.5 µM IAA + 30 µM ADS	Sharma and Shahzad (2012)
Withania somnifera	Nodal segments with axillary buds	4 °C	2–8 weeks	50.5–86.2	86.2% after 4 weeks	MS + 2.5 µM BA + 0.5 µM NAA	Fatima et al. (2013)
Hemidesmus indicus	Somatic embryos	4 °C	4 months	86–100	90% after 90 days	MS + 2 µM Kn + 0.5 µM IBA	Cheruvathur et al. (2013)
Cassia angustifolia	Nodal segments	4 °C	0–8 weeks	43.90–94.06	72.30% after 4 weeks	MS + 2.5 µM BA + 0.4 µM NAA	Parveen and Shahzad (2014)
Sterculia urens	Nodal segments	4 °C	0–6 months	73.33–95	73.33 after 6 months	MS + 0.2 mg L^{-1} TDZ	Devi et al. (2014)

Ocimum kilimandscharicum	Shoot tip	4 and 25 °C	30–60 days 30–90 days	23.60–9.72 54.16–81.94	81.94% at 25 °C after 30 days	MS + 1.0 mg/L BA	Saha et al. (2015)
Vitex trifolia	Nodal segments	4 °C, 15 °C, and 24 °C	0–8 weeks	42.5–92.3	74.5% after 4 weeks	MS + 5.0 μM BA + 0.5 μM NAA	Ahmed et al. (2015)
	Nodal segments	4 °C	2–12 weeks	48–97	71.6% after 8 weeks	MS + 2.5 μmol/L KN + 1.0 μmol/L NAA	Alatar et al. (2017)
Curcuma amada	Somatic embryos	4 °C and 25 °C	0–120 days	54.16–86.11 37.49–72.22	88.10% at 4 °C after 30 days	½ MS + 0.25 mg L^{-1} GA_3	Raju et al. (2016)
Urginea altissima	Shoot tips	4 and 25 ± 2 °C	15 days	67.6% at 4 °C and 91% at 25 ± 2 °C	91% at 25 ± 2 °C	MS + 10 μM *m*T + 2 μM NAA	Baskaran et al. (2017)
Spathoglottis plicata	Protocorm-like bodies	4 °C, 15 °C, and 24 °C	0–120 days	48–88%, 27–88%, and 18–88%	66.7% after 90 days at 4 °C	MMS + 0.5 mg L^{-1} Kin	Haque and Ghosh (2017)
Capparis decidua	Nodal segments	4 °C	0–6 weeks	73–93	93% after 4 weeks	MS + 5.0 μM TDZ + 0.5 μM IAA	Siddique and Bukhari (2018)
Salix tetrasperma	Nodal segments	4 °C	0–8 weeks	49.67–91.33	71% after 4 weeks	WPM + 2.5 μM Kin + 0.5 μM NAA	Khan et al. (2018)
Rosa damascena trigintipetala	Axillary buds	4 °C	0, 4, and 8 weeks	60–90	60% after 8 weeks	MS + 0.5% sucrose	Attia et al. (2018)