Synthetic Seeds of Wild Beet: Basic Concepts and Related Methodologies

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Abstract Synthetic seeds are artificially encapsulated propagules that mimic true seeds in agriculture. Although a variety of plant materials, such as shoot tips, axillary buds, callus, micro cuttings, and protocorm-like bodies, are used in the production of synthetic seeds, somatic embryos are the most widely used explants in the production of these seeds. Synthetic seeds compete with traditional approaches to preserve the germplasm of threatened plant species. The resulting progenies are the true clones of the main plant, thus preserving the intactness of the genetic background. Due to poor germination and low seed amount, wild *Beta* species are exposed to the risk of extinction. Wild relatives of *Beta* have agronomically important properties such as resistance to diseases and abiotic stresses. Numerous attempts have been made to give these traits to sugar beet crop through conventional breeding methods. Despite the importance of synthetic seed for wild beets, it has not yet been investigated. The production of synthetic seeds ensures the conservation and availability of wild germplasm of the genus *Beta* for cytogenetic and breeding studies.

Keywords Artificial seeds · Biotic and abiotic stress · Genus *Beta* · Germplasm conservation · Resistance genes

1 Introduction

Seeds are zygotic embryonic plants produced after fertilization in flowering plants. Seeds connect different generations of plants and ensure the maintenance and transfer of plant genetic material in nature. These structures are enclosed with protective layers to keep the embryo safe during storage and dispersal (Bewley and Black 1985).

Seeds are essentially composed of an embryo and protective layers. True seeds contain endosperm tissues that provide the nutrients necessary for germination. Endosperm stores various substances mainly including starch, proteins, and oils.

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However, in some species, cotyledonary leaves are the source of these components. Seeds are the basis of agriculture and, when the necessary factors for germination are provided, they produce a plant similar to the main plant.

Depending on plant species and conditions, the production of true seeds can be time-consuming, arduous, impossible, or costly. Synthetic seeds have great potential as an alternative to true seeds, especially due to low production costs and long-term storage (Roy and Mandal 2008). These seeds can be handled, stored, transported, and planted like true seeds (Sharma et al. 2013).

The term "synthetic seed" was first defined as an encapsulated single somatic embryo by Murashige (1977). In the early studies, the concept was limited to plants where somatic embryogenesis could be demonstrated; however, it was later on extended to any in vitro-derived propagule due to the recalcitrance of some species to somatic embryogenesis (Bapat et al. 1987). Synthetic seeds are also known as artificial seeds, synseeds, and manufactured seeds. Somatic embryos are often used as propagule in the production of synthetic seeds; however, cell aggregates, shoot buds, auxiliary buds, or any other structure capable of giving rise to a plant can also be used. Furthermore, these seeds can hold this ability for a long time and can be stored (Ara et al. 2000; Saiprasad 2001; Daud et al. 2008).

Synthetic seed technology is a suitable alternative method for proliferation of commercially important plants. These tissue culture-originated artificial seeds are economically preferred for the propagation of hybrids generated through breeding approaches (Rihan et al. 2017). Synthetic seeds, however, can be used to proliferate the species that hardly reproduce through generative propagation and are at risk of extinction.

The wild relatives of sugar beet (*Beta vulgaris* spp. *vulgaris*) are invaluable germplasm reservoirs that are in danger of extinction. Although synthetic seeds have been produced for more than 30 years, no attempt has been made to utilize synthetic seed technology for wild beet species. However, in situ and ex situ efforts have been made to protect these invaluable genotypes (Frese et al. 2001).

In this chapter, we emphasize the importance of wild beet relatives in the genus *Beta* and review the synthetic seed technology to evaluate the use of this approach for wild beet accessions.

2 Origin and Development of Synthetic Seeds

When Steward et al. (1958) and Reinert (1958), at the same time, reported the first somatic embryogenesis in the carrot plant (*Daucus carota*), most probably the idea of synthetic seed production created. However, the first report of plant propagation by somatic embryos was presented by Murashige (1977). He succeeded to obtain the surviving plants originating from somatic embryos in vitro conditions. Studies continued to accelerate the process of developing plants from somatic embryos for commercial applications. Drew (1979) delivered the carrot somatic embryos in a liquid drilling system; however, the results were disappointing as he developed only three carrot plants in carbohydrate-free media. The first synthetic seed production goes back to a report by Kitto and Janick (1982). They encapsulated multiple

somatic embryos of carrot in a polyoxyethylene glycol and desiccated the embryos. Polyoxyethylene is a readily soluble chemical in water and after drying forms a thin layer. In addition, it is not toxic to the embryo and does not allow growth of the microorganism and, therefore, later was also used to encapsulate the celery (*Apium graveolens*) embryos (Kitto and Janick, 1985). A polyethylene glycol (PEG)-based mixture was also applied to coat the carrot somatic embryos and embryonic calli. Following the dehydration procedure, the survival rate was scored by placing the coated embryos on culture medium allowing them to rehydrate (Janick et al. 1993). Since the first studies, many efforts have been made to produce synthetic seed in various plant species (Ravi and Anand 2012). Survival percentage of encapsulated embryos was enhanced by modifications applied in the encapsulation matrix and speed of dehydration. Various propagules have been utilized to establish the system for the species recalcitrant to somatic embryogenesis.

3 Types of Synthetic Seeds

The somatic embryos used for the production of synthetic seeds may or may not be encapsulated. The state of quiescence in uncoated somatic embryos defines the usage of these seeds. If the uncoated embryo is non-quiescent, it is used for the in vitro micropropagation of plant, and if quiescent, it is used for the germplasm storage. The encapsulated somatic embryos are divided into two types of hydrated and dried seeds. The encapsulated somatic embryos may be non-quiescent or quiescent if the seed coat is hydrated or desiccated, respectively. The quiescent somatic embryos coated with dehydrated artificial coatings are the most resembling synthetic seeds to the conventional ones in terms of handling and storage.

The vigor of the seedlings obtained from the desiccated embryos is higher than that obtained from the hydrated embryos. It is ideal if the synthetic seed is produced through encapsulated desiccated embryos (Pond and Cameron 2003). Prior to encapsulation process, somatic embryos are hardened to tolerate the desiccation which induces the quiescence. However, desiccated seeds are only produced if the somatic embryos are tolerant to dehydration. In some species, the generated somatic embryos are sensitive to water deficiency; thus, the embryo ought to be coated with hydrogels (Magray et al. 2017). Desiccation degree is determinative and depends on the developmental stage of the embryo. If the embryo is mature, the drying process should be carried out rapidly, and if it is immature, it is opted to implement the process slowly (Senaratna et al. 1990).

4 Wild Beets: Synthetic Seed and Applications

4.1 Species of the Genus Beta

Based on the crossing ability between cultivated beet and other genotypes, the genus *Beta* divides into three gene pools: the primary gene pool contains the cultivated beet

and other species which can easily cross; the secondary gene pool includes the species that despite the crossability with cultivated beet generate sterile progenies; and the tertiary gene pool members generate hybrids only by human intervention and artificial crossing (Jassem 1992; Kadereit et al. 2006; Frese 2010) (Table 1). However, an earlier classification had placed *B. nana* in a separate section, thus dividing the genus *Beta* into four sections as *Beta*, *Corollinae*, *Nanae*, and *Procumbentes* (Ford-Lloyd 2005). The Eastern Anatolia-Western Caucasus crossing region is accepted as the origin and resource of genetic diversity for *Beta* and *Corollinae* (Boughey 1981). Section *Nanae* is distributed in the mountainous regions of Greece, whereas the members of the section *Procumbentes* spread far away in the Canary Islands, coastal areas of North-West Africa, and Southeastern Spain (Frese 2010).

4.2 Employment of the Wild Beet Genotypes

The interest in wild beet genotypes as the genetic resources for improving the sugar beet germplasm has been increasing since the late 1800s, and the values of these genotypes have been well-demonstrated (De Bock 1986; Doney and Whitney 1990; Van Geyt et al. 1990; Lewellen and Skoyen 1991; Doney 1993). Since that time, breeders have been employing these germplasms to improve the agronomic traits of the sugar beet crop. There are committees established to study and protect the germplasm of the genus *Beta*. Very comprehensive information on the genotypes of *Beta* is available at the Genetic Resources Information Network (GRIN) Database of National Plant Germplasm System (NPGS). More than 22% of the available *Beta* accessions are the *B. maritima* accessions which are well-characterized and most useful accessions in the breeding of sugar beet (Biancardi et al. 2010).

4.3 Wild Beets Are the Reservoir for Resistance Genes

Sugar beet yield is affected by different biotic and abiotic factors. The cyst nematode (*Heterodera schachtii* Schm.), leaf spot disease (*Cercospora beticola* Sacc.), and *Beet necrotic yellow vein virus* (BNYVV) are among the most destructive pests and disease agents of sugar beet crop that reduce root yield and sugar content (Weiland

Table 1 Gene pools of the genus *Beta* (Jassem 1992; Kadereit et al. 2006; Frese 2010)

Gene pool 1	Gene pool 2	Gene pool 3
Beta vulgaris ssp. *vulgaris*	*Beta corolliflora*	*Beta patellaris*
Beta vulgaris ssp. *adanensis*	*Beta macrorhiza*	*(Patellifolia patellaris)*
Beta vulgaris ssp. *maritima*	*Beta lomatogona*	*Beta procumbens*
Beta macrocarpa	*Beta trigyna*	*(Patellifolia procumbens)*
Beta patula	*Beta intermedia*	*Beta webbiana*
	Beta nana	*(Patellifolia webbiana)*

and Koch 2004; McGrann et al. 2009; Biancardi et al. 2010). Sugar beet cyst nematode is spread over more than 40 countries and causes major product losses (McCarter 2008). However, some *Beta* species carry cyst nematode resistance genes that can be employed in breeding programs. *C. beticola* is a fungal pathogen known as the most damaging sugar beet disease worldwide, corresponding to leaf spot disease in sugar beet (Weiland and Koch 2004). Depending on the severity of the disease, *C. beticola* reduces the sugar yield by 1–55% (Rossi et al. 1995; Altınok 2012). The soil-borne disease of rhizomania is caused by BNYVV, which is transmitted by *Polymyxa betae* Keskin (Keskin 1964). Rhizomania causes yield losses of up to 80% in the sugar beet crop and the only way to control the disease is to cultivate resistant varieties (Tamada and Baba 1973).

Wild relatives of genus *Beta* contain agronomically important characteristics and can be exploited in introgression of desirable traits into cultivated beet crop (Table 2). Being a member of the section *Beta*, *B. v.* ssp. *maritima* is a very close and cross-compatible relative of *B. v.* ssp. *vulgaris* (Biancardi et al. 2012). It owns valuable traits such as resistance to *H. schachtii* and is the widely deployed wild-type genotype in sugar beet breeding studies (Panella and Lewellen 2007). In addition, *B. maritima* is a source of resistance to *Cercospora leaf spot* disease, so it has been used to develop resistant varieties (Munerati et al. 1913). Although resistance to *Cercospora* has been reported in all of the *Corollinae*, *Procumbentes*, and *Beta* sections, the resistance degree is highly different between them. Among species, *B. maritima* has the most pronounced degree of resistance, so it is widely used in sugar beet breeding approaches (Munerati 1932; Panella and Frese 2000; Skaracis and Biancardi 2000; Luterbacher et al. 2004). In contrast to *B. maritima*, *B. nanae* and *B. macrorhiza* accessions are susceptible to *Cercospora* (Coons 1954). *B. maritima* also could be a reliable resource for resistance genes of rhizomania, and among these genes, *Rz1* and *Rz2* are generally used in the commercial development of resistant varieties (Scholten et al. 1999). Wild beet genotypes are also invaluable resources of tolerance to abiotic stresses such as drought, salt, and frost which could be exploited to improve the quality of growth in sugar beet under adverse climatic conditions (Frese et al. 2001). The *Corollinae* section, in particular *B. corolliflora*, is thought to be an important germplasm against abiotic stresses. However, the low homology percentage of chromosomes between different gene pools may be an obstacle in the transmission of the desired genes (Jung and Wricke 1987; Van Geyt et al. 1990).

4.4 The Contribution of Synthetic Seeds to Breeding Programs

The first production of the sugar beet crop was restricted to Northern Europe, a temperate climate and a relatively disease-free zone; therefore, small selection pressure was applied against the pathogens. Following the cultivation in other

Table 2 Transition of resistance genes from wild beet genotypes to sugar beet crop via conventional breeding approaches

	Resource of resistance	*B. vulgaris* hybrid accessions obtained through interspecific breeding	Biotic factor	References
Beta section	*B. maritima*—WB42, Rızor or Holly, WB41, WB258, R36, R22 (PI 590791)	*B. vulgaris*—Holly-1–4, R104	BNYVV	Lewellen and Whitney (1993), Pelsy and Merdinoglu (1996), Scholten et al. (1999), Gidner et al. (2005), Grimmer et al. (2008a, b)
	B. maritima	*B. vulgaris*—CN921-515 (Reg. No. GP-295, PI 669447) and CN921-516 (Reg. No. GP-296, PI 669448)	Cyst nematode	Richardson (2018)
	B. maritima—WB242 (PI 546413), N499 (PI 599349)	*B. vulgaris*—CN12 (PI 636338), CP07 (PI 632288), CP08 (PI 6322889)	Cyst nematode	Heijbroek et al. (1977), Lewellen (2004, 2006)
	B. maritima	*B. vulgaris*—PI 357354, PI 518303, PI 546413, PI 504180, and PI 546413	Cyst nematode	Panella and Lewellen (2007)
	B. maritima	*B. vulgaris—M66, WB258 (PI 546426)*	Root-Knot Nematode	Yu (1997, 2002)
	B. maritima	*B. vulgaris*—Rovigo (R148, R581, etc.), varieties "Cesena" and "Mezzano"	*Cercospora*	Munerati (1932), Biancardi and De Biaggi (1979)
	B. maritima—WB97, WB242	*B. vulgaris*—CP01 and CP02	Powdery mildew	Lewellen (2000)
	B. maritima—WB178, PI 546403	*B. vulgaris*—83 W304	Yellow wilt	McFarlane (1984)
	B. maritima—PI 546409 (WB185), PI 540625 (WB879)	*B. vulgaris* (SP6822 X WB879)	*Aphanomyces*	Yu (2004)

(continued)

Table 2 (continued)

	Resource of resistance	*B. vulgaris* hybrid accessions obtained through interspecific breeding	Biotic factor	References
Procumbentes section	*B. procumbens*	*B. vulgaris* (B883, ANl-65-2, AN101)	Cyst nematode	Van Geyt et al. (1988), Lange et al. (1988, 1993), Salentijn et al. (1992)
	B. procumbens—AU6-1-4 and D3-2-13; *B. patellaris* – B1-1-54	*B. vulgaris*—Holly-1–4	*Cercospora*	Mesbah et al. (1997)
	B. patellaris—A5-1-7 and B1-1-192	*B. vulgaris*—Holly-1–4	BNYVV	Mesbah et al. (1997)

regions with different climatic conditions, various diseases emerged and affected crop yield (Lewellen 1992). Although numerous genetic improvements have been made to date, susceptibility to disease still threatens crop yields and production worldwide (Table 2). Wild beet accessions have great potential to expand the germplasm pool of sugar beet. Wild *Beta* species are evaluated in terms of important agronomic characteristics to be used in sugar beet breeding. These accessions indicate different degrees of resistance to biotic and abiotic factors. Initial efforts to screen wild beet genotypes for pathogen resistance were made in the early 1900s. The first documented report of using the *B. vulgaris* ssp. *maritima* in breeding studies of cultivated beet belongs to Munerati et al. (1913) who found *B. maritima* as a source of resistance to *Cercospora leaf spot* disease (Biancardi et al. 2010). However, various resistance genes were later on transferred to *B. vulgaris* through interspecific hybridization. The obtained interspecific hybrids were resistant to different biotic factors (Table 2). The synthetic seed technology contributes to the breeding of cultivated accessions. The preparation of synthetic seeds for *Beta* wild relatives ensures the presence of these accessions for breeding programs.

Sugar beet is a biennial crop and has to be exposed to the prolonged cold of winter for flowering and seed production (Letschert 1993). However, wild-type relatives are naturally annual, and production of the flowering stem is triggered in the first year (Biancardi et al. 2010). Depending on the geographical origin and altitude, a perennial growth pattern is also possible in wild beet genotypes (Marlander et al. 2011). In vitro production of synthetic seeds allows researchers to shorten the breeding process by applying equivalent but controlled conditions to stimulate vernalization.

Simultaneous flowering time is very important for successful plant-pollinator interactions (Elliott and Weston 1993; Alcaraz et al. 1998). The lack of synchronicity in flowering, especially in different genotypes, makes the hybridization of wild

and cultivated species difficult (Alibert et al. 2003; Cuguen et al. 2005). Application of synthetic seeds and human intervention to adjust the cultivation time enhance the chance of fertilization and seed production. The mass production of clonal plants with homogeneity in the genetic background causes simultaneous flowering in the population. In addition to homogeneity in flowering, the production of large amounts of pollen is another feature that breeders will appreciate when they intend to convey the desired characteristics between species. Compared to the monogerm seeds, the multigerm *Beta* accessions produce more pollen (Alibert et al. 2003; Biancardi et al. 2010). Therefore, the selection of superior genotypes that match the breeding objectives is an important step before hybridization.

The production of inbred lines is necessary for the development of hybrid seeds. However, inbreeding is sometimes not possible due to genetic barriers and the presence of allogamy in many species. Sugar beet is primarily self-incompatible, and the self-pollination is rare among wild beets. This feature was used by breeders to increase and maintain heterosis in multigerm varieties prior to the discovery of cytoplasmic male sterility (Owen 1942). Large-scale conservation and micropropagation of selective rare hybrid genotypes have attracted interest in the production of synthetic seeds. The production of synthetic seeds can eliminate the genetic segregation because of the participation of only one parent, as well as the use of somatic cells in seed production. These seeds are the actual clones of the sampled plant, so it can also be considered in the maintenance of the unstable sterile genotypes, genotypes that have difficulty in germination and transgenic plants (Gantait et al. 2015).

Hybridization of species distributed in different gene pools is limited due to existing genetic barriers (Abe and Tsuda 1987; Jung and Wricke 1987; Van Geyt et al. 1990). However, there are successful hybridization reports between the species of different sections (Table 2). The obtained interspecific hybrids might lack one or more chromosomes leading to aneuploidy. These individuals will be used in cytogenetic and biotechnological studies of *Beta* species (Savitsky 1960, 1975; Yu 1983, 2005; Heijbroek et al. 1988; Sandal et al. 1997). Thus, the protection of the desired genotypes between such hybrids requires an asexual multiplication technique. Artificial seed technology can protect and reproduce the desired progeny for future uses in breeding programs.

5 Germplasm Conservation of Wild Beet Species

True seeds are consisting of an embryo, nutritive tissues, and protective layers. The embryo and nutritive tissues of true seeds are covered with a seed coat which keeps the embryo quiescent and tolerant to adverse climatic conditions that have been the source of inspiration for the preservation of the germplasm in gene banks. Zygotic seeds are reliable sources to preserve germplasms in repositories. Furthermore, the incidence of pathogenic infestations and high metabolic activities affect the period of seed storage. Unlike the true seeds, synthetic seeds do not necessarily contain nutritive tissues or seed protective layers and the state of quiescence might be

different in these seeds. Therefore, depending on the purpose of use, the structural complexity of synthetic seeds is defined. Seed coats of synthetic seeds not only help to the keep the propagules from pathogenic diseases but also establish a safe seedbed in the soil. It protects the propagules from drought and other unfavorable conditions and also keeps the seed safe during the transportation and storage (Ara et al. 2000).

True seeds of the wild beet species are coated with a thick and excessively indented testa which causes poor germination (Coons 1975). The perennial pattern of growth and the low germination rate affect the multiplication of wild beet relatives in natural habitats (McGrath et al. 2007; Marlander et al. 2011). The difficulties in germination and proliferation, especially among the species of *Corollinae*, challenge the survival of wild beet species (Frese et al. 2001). To date, in situ and ex situ efforts have been made to save these genotypes in their natural distributed areas or botanical gardens (Ren et al. 2012). However, the need to accelerate and improve the propagation process and efficiency is still felt.

Unlike the true seeds which produce following the sexual reproduction and undergo the genetic recombination process, the intactness of the parental genetic background is protected in synthetic seeds. Propagules used in the production of synthetic seeds are generated in aseptic conditions; therefore, the resulting seeds are pathogen-free and thus are superior to the traditional methods in which diseases encounter severe threats to the stoked genotype. Moreover, the international exchange of plant material would be easier and faster across the country borders and successfully contribute to the control of plant diseases (Daud et al. 2008; Nyende et al. 2005). Recently the in vitro multiplication of wild beet genotypes was investigated to assess the in vitro regeneration potential of different species of the genus *Beta* (Ergül et al. 2018). Results indicated the multiplication capability of the selected genotypes when were subjected to cytokinin and gibberellin growth regulators. In an early study, the callus induction potential of the wild genotypes of *Beta* sections was evaluated (Yu 1989). Despite the conducted studies, the assessment of somatic embryogenesis in these species yet remains. Somatic embryos are frequently used as proper explants in the production of synthetic seeds; therefore, the importance of studies over the somatic embryogenesis in wild beet species is emphasized (Skaracis 2005).

Production of synthetic seeds will provide a sufficient quantity of wild beet accessions to ensure the germplasm conservation. This technique will ease the renewal of germplasm in gene banks, and the germplasm continuity will be ensured; moreover, the costs of germplasm maintenance in the gene banks will be reduced efficiently.

6 Synthetic Seed Production and Storage Methods

6.1 Explant Materials

A variety of propagules have been utilized in the production of synthetic seeds (Fig. 1). Propagules are divided into two categories of unipolar and bipolar. Both

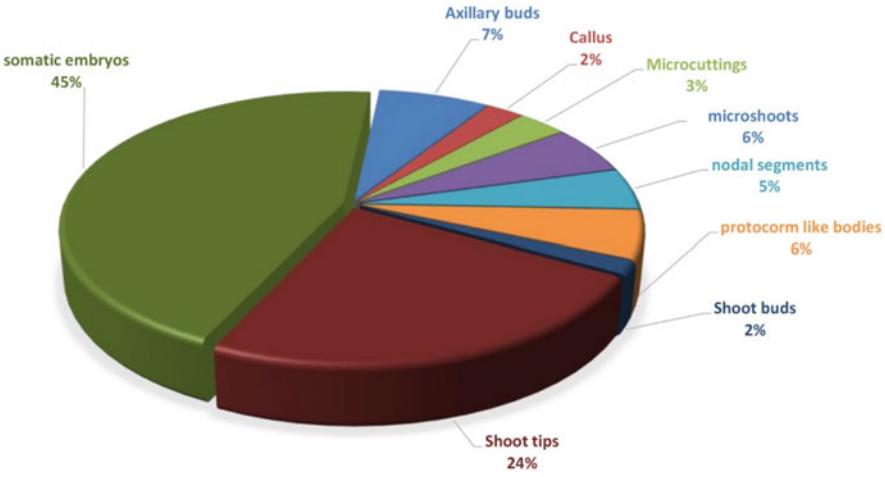

Fig. 1 The pie chart depicts the propagules used in synthetic seed production and their relative contribution to synthetic seed production studies (Reddy et al. 2012)

types are used in the production of synthetic seeds and have pros and cons. Bipolar propagules such as somatic embryos contain shoot and root apical meristems and generate the plant in a single stage (Ara et al. 2000). Such seeds can be used as hydrated or dried. Seeds carrying the somatic embryos have a strong capacity for reproduction and can maintain their regenerative potential for a long time leading to a uniform plant production (Leroy et al. 2000). The germination rate of the somatic embryos is different from that of zygotic embryos so that the zygotic embryos are superior to synthetic seeds in germination rate (Lulsdorf et al. 1993; Attree et al. 1994). Nevertheless, the encapsulation method and the plant genotype are determining factors in germination rate of the seeds (Cartes et al. 2009). The resulting plants originating from bipolar propagules may cause somaclonal variations. Moreover, asynchronous and late maturation features of embryonic poles are among the factors restricting the usage of somatic embryos in the production of synthetic seeds (Castellanos et al. 2004). On the other hand, unipolar propagules contain only a shoot or a root pole and are superior to the bipolar ones in terms of genetic fidelity and a vast range of vegetative explants. Unipolar explants can be any of the apical tips, axillary buds, micro shoots, micro bulbs, microtubers, corms, rhizomes, meristemoids, cell aggregates, and primordia biological materials with different levels of complexity and conversion ratios (Sharma et al. 2013).

Although propagules such as shoot apical meristems, axillary buds, and micro shoots do not contain root apical meristem, these are encapsulated to generate seeds. However, prior to the encapsulation process, explants are subjected to the root inducing chemicals. Some studies reported the possibility of root induction and conversion of the buds to plantlets when cultivated on white's rooting medium without chemical pretreatments (Bapat and Rao 1990; Ganapathi et al. 1992). Based on a study, the conversion of the encapsulated apical tips was more than

axillary buds; however, a later study indicated the conversion of 100% for axillary buds when encapsulated in a suitable matrix (Capuano et al. 1998; Lata et al. 2009).

Plant species respond differently to synthetic seed production that emphasizes the effect of genotype and plant species. The literature review shows that somatic embryos as the most corresponding propagule have been employed in the majority of the studies including vegetable crops, spices, and plantation crops, ornamental plants and orchids, medicinal plants, forage legumes, fruit crops, and forest trees (Reddy et al. 2012). Following somatic embryos, shoot tip explants are the second most widely used propagule; however, other explant sources have been less frequently used (Fig. 1). Both somatic embryos and shoot tip explants were used as plant material in the production of synthetic seeds for *B. vulgaris*, which can also be recommended for wild relatives of *Beta* (Saunders and Tsai 1999; Rizkalla et al. 2012).

6.2 Encapsulation Chemicals and Processes

Agents such as agar, agarose, alginate, polyox, polyco 2133, guar gum, tragacanth gum, gelrite, carrageenan, carboxymethyl cellulose, polyacrylamide, nitrocellulose, and sodium pectate ethyl cellulose have been tested for the encapsulation of synthetic seeds till date (Ara et al. 2000; Saiprasad 2001; Lambardi et al. 2006). Among these chemicals, sodium alginate has been found as the most suitable agent for encapsulation of somatic embryos due to its low cost, low toxicity, and quick gelling properties (Saiprasad 2001). This chemical was used to encapsulate the *B. vulgaris* somatic embryo and shoot tip propagules at concentrations of 2% and 4%, respectively (Saunders and Tsai 1999; Rizkalla et al. 2012). Sodium alginate can protect the biologic material for a longer time when compared to other agents such as agar. The firmness of the seed coat is determined by the ratio of sodium ions exchanged with calcium in $CaCl_2 \cdot H_2O$ solution (Daud et al. 2008). The literature review indicates that the most satisfying results are obtained once explants are encapsulated with 3% sodium alginate and 100 mM $CaCl_2 \cdot H_2O$. In the majority of these studies, the regeneration frequency was noted more than 90% (Singh and Chand 2010; Ahmad et al. 2012; Sakhanokho et al. 2013; Varshney and Anis 2014).

Structure of the synthetic seeds imitates that of true seeds. The biologic material in synthetic seeds represents the zygotic embryo in true seeds (Cartes et al. 2009). Synthetic endosperms are comprised of MS culture medium supplemented with growth regulators such as cytokinin and auxins, minerals and vitamins, gelling chemical, and anti-pathogenic components (Ravi and Anand 2012).

Synthetic seeds are single-layered, double-layered, or hollow bead structures. To produce single-layered seeds, the in vitro originated plant materials are mixed with a proper hydrogel. Alginate is the most frequently used coating agent employed in the concentration of 0.5–5%. After alginate dissolves in double distilled water or liquid nitrogen, the solution is utilized in the production of the beads containing propagules. The beads are then treated with a complexion agent such as calcium chloride ($CaCl_2 \cdot 2H_2O$). It is important to obtain round and firm calcium alginate beads.

The concentration of sodium and calcium together with the complexion time affect the permeability and rigidity of the beads and may be different between plant species. Generally, treatment of beads with 3% (w/v) sodium alginate and 100 mM calcium chloride for 20–30 min is reported as the most suitable combination for seed production (Sarkar and Naik 1998; Tabassum et al. 2010; Ahmad and Anis 2010; Ozudogru et al. 2011; Alatar and Faisal 2012; Hung and Trueman 2012a, b). Low alginate concentrations (<3%) interfere with solidification and high concentrations (5–6%) result in a very hard coating that delays germination (Larkin et al. 1998; Ahmad and Anis 2010; Sharma et al. 2009a, b).

The content of the matrix, such as nutrients and growth regulators, influences the success of germination and conversion of the encapsulated explant (Chand and Singh 2004; Sundararaj et al. 2010). Synthetic seeds are highly susceptible to microbial infections, so various antimicrobial agents are added to the gel matrix to reduce infection (Saiprasad 2001; Wang et al. 2007).

The addition of activated carbon to the matrix gel enhances explant conversion and vigor. Activated carbon adsorbs toxic products such as phenolic compounds that can damage encapsulated propagule; moreover, it helps the diffusion of nutrients and gases. It contributes to decomposition of alginate and enhanced respiration of the biological material, thereby prolongs the storage time. Additionally, the activated carbon retains the nutrients and releases them gradually which provides a long-term supply of essential nutrients for the propagule. Pretreatment of synthetic seeds with potassium nitrate makes an impressive contribution to the production of shoots and roots from coated propagules (Sharma et al. 2013).

Double layering the plant material is proposed to increase the protection of encapsulated propagules. Once the single layer seeds are produced, they can be coated with the same concentration of sodium alginate and then coated with the treatment of $CaCl_2 \cdot 2H_2O$. Double-layered synthetic seeds have the same properties of the single-layered ones; however, double encapsulation provides better protection (Micheli et al. 2002; Pinker and Abdel-Rahman 2005).

When the position of the propagule in the bead is compared between conventional synthetic seeds and true seeds, the adjacent position of the propagule to bead surface makes the protection fragile in synthetic seeds. Hollow beads are promising tools to assure the protection of encapsulated propagules; however, the process is laborious and costly, and the success of this method is still controversial (Winkelmann et al. 2004; Pourjavadi et al. 2006).

6.3 Storage of Synthetic Seeds

Storage of synthetic seeds is carried out for a variety of purposes, such as the germplasm transport between countries, proliferation, and protection of invaluable germplasms. Researchers investigate the ideal conditions for the storage of synthetic seeds. Storage temperature and matrix components are the most important factors affecting the conversion rate; however, the effect of species on the storage is also

decisive. Generally, 4 °C is the most suitable temperature for short-term storage of synthetic seeds including *B. vulgaris* (Saiprasad and Polisetty 2003; Kavyashree et al. 2006; Singh et al. 2007; Faisal and Anis 2007; Pintos et al. 2008; Sharma et al. 2009a, b; Ikhlaq et al. 2010; Tabassum et al. 2010). However, several studies on some tropical and subtropical crops have reported that higher temperature (25 °C) is required for bead storage (Srivastava et al. 2009; Sundararaj et al. 2010; Mishra et al. 2011). Although wild *Beta* species have not been investigated for synthetic seeds, there are reported studies over *B. vulgaris* spp. *vulgaris*. In vitro storage of sugar beet synthetic seeds was evaluated after addition of osmotic agents to the MS medium (Rizkalla et al. 2012). According to the results, the addition of 0.05 M mannitol or sorbitol to the medium increased seed survival during in vitro storage but disrupted growth quality. This result is consistent with Westcott (1981), which previously reported the toxic effects of mannitol. In an early study, the effect of cold storage of the beads was investigated, and the results showed that the cold treatment did not improve the conversion rate and also slowed down the rate of development (Saunders and Tsai 1999).

For long-term storage of synthetic seeds, dehydration and cryopreservation storage techniques are used. Cryopreservation of the propagules can only be useful if the formation of intracellular ice crystals is avoided; otherwise, harmful effects prevent cell survival. Various techniques of cryopreservation inclusion of simple desiccation, encapsulation-dehydration, the two-stage freezing, vitrification, and encapsulation-vitrification have been employed to date. Pretreatment of encapsulated biological tissues in a medium supplemented with high concentrations of sucrose results in progressive water withdrawal of the coated propagule. Additional dehydration of the encapsulated explants increases the concentration of sucrose results in a glass transition during cooling to ultralow temperatures (–196 °C) and subsequently fatal damages to the cells (Engelmann and Takagi 2000). Figure 2 summarizes the approaches used to store synthetic seeds.

7 Difficulties in the Production of Synthetic Seeds

Large-scale and cost-effective synthetic seed production requires investigations of high-quality propagules and encapsulation methods. Since the first synthetic seed production report, many improvements have been made. Despite the impressive advantages, this technique is currently facing limitations that challenge commercial production. Lack of dormancy in synthetic seeds causes limitations during the storage period. Especially because these seeds are stored at lower temperatures, the vitality and conversion rate reduces over time. Among the biological materials studied, somatic embryos are superior to others because of their potential in the production of the shoot and root system in one step. However, in addition to improper maturation of somatic embryos, synchronic deficits in the development of somatic embryos make the application of somatic embryos difficult (Reddy et al. 2012; Hung and Trueman 2012a, b). Somatic embryogenesis may be difficult due to the existing recalcitrance of

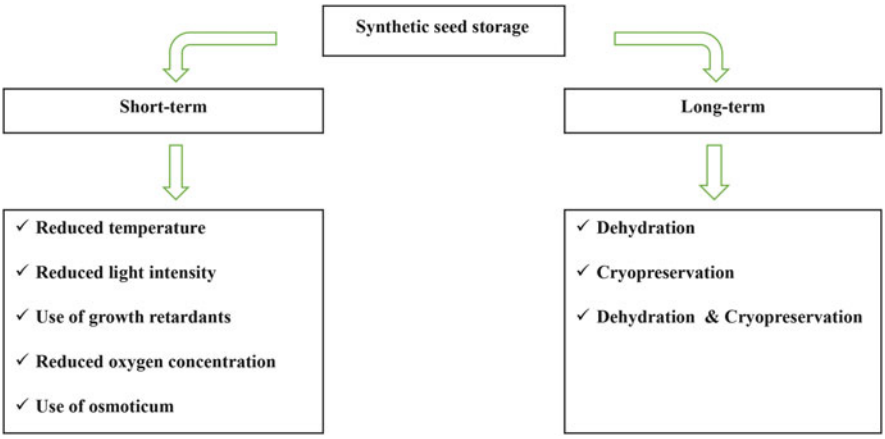

Fig. 2 Various techniques are used to store synthetic seeds depending on the purpose of storage

some plant species, thus encouraging the use of other explant sources in the production of synthetic seeds. Although such explants are promising tools, the creation of the root system is a complex challenge in this approach. In most woody plant species, single-step rooting is the major obstacle to non-embryogenic coated propagules (Chand and Singh 2004; Naik and Chand 2006; Hung and Trueman 2012a).

One of the main problems of synthetic seeds is the practicality of these seeds during sowing under ex vitro conditions. In addition to the oxygen supply and nutrient deficiency, the presence of various pathogens in commercial substrates such as soil or vermiculite causes infection risks and other limitations (Jung et al. 2004; Rihan et al. 2012). However, the successful conversion of encapsulated propagules into vigorous seedlings remains one of the important factors that hamper commercial production (Sharma et al. 2013). Adjusting the matrix composition of the beads will overcome the barriers that exist in storage and direct sowing. The hydrated calcium alginate-based or dried polyethylene glycol-based encapsulation will also provide the benefit of synthetic seed technology for long-term preservation of germplasms by cryopreservation (Ara et al. 2000).

The in vitro responses of wild beet species are not well studied yet. For instance, the somatic embryogenesis capability of these species is still unknown, whereas somatic embryo is the most widely used explant in synthetic seed production. Lack of in vitro studies along with frequently encountered difficulties may cause similar risks in the artificial seed production of wild *Beta* species.

8 Conclusion

Synthetic seeds have the potential to take part in the protection of endangered germplasms or to produce economic plants on commercial scales. Despite the importance of wild beet genotypes, synthetic seed technology for these species has

not yet been investigated. The development of in vitro protocols for somatic embryogenesis of these species is particularly important in the first step. The encapsulation procedure, as well as storage, should be examined for wild beet accessions.

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