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Research review paper

Synseed technology—A complete synthesis

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ABSTRACT

Progress in biotechnological research over the last two decades has provided greater scope for the improvement of crops, forest trees and other important plant species. Plant propagation using synthetic seeds has opened new vistas in the field of agriculture. Synseed technology is a highly promising tool for the management of transgenic and seedless plant species, polyploid plants with elite traits and plant lines that are difficult to propagate through conventional propagation methods. Delivery of synseeds also alleviates issues like undertaking several passages for scaling up *in vitro* cultures as well as acclimatization to *ex vitro* conditions. Optimization of synchronized propagule development followed by automation of the whole process (sorting, harvesting, encapsulation and conversion) can enhance the pace of synseed production. Cryopreservation of encapsulated germplasm has now been increasingly used as an *ex vitro* conservation tool with the possible minimization of adverse effects of cryoprotectants and post-preservation damages. Through synseed technology, germplasm exchange between countries could be accelerated as a result of reduced plant quarantine requirements because of the aseptic condition of the plant material.

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1. Introduction

An active research front has emerged over the last decade with the goal of developing non-zygotic embryogenesis into a commercially useful method of plant propagation. From Haberlandt's postulate (1902) of artificial embryo cultivation to the concept proposed by Murashige (1977), artificial seeds have evolved from a futuristic idea into a real field of experimental research. The term "artificial seed", which was first coined by Murashige, is now also known by other names including manufactured seed, synthetic seed or synseed. The original definition of an artificial seed, as given by Murashige (1978), was "an encapsulated single somatic embryo", i.e., a clonal product that could be handled and used as real seed for transport, storage and sowing and that, therefore, would eventually grow either *in vitro* or *ex vitro*, into a plantlet ("conversion"). Gray and Purohit (1991) also defined synseed as "a somatic embryo that is engineered for the practical use in commercial plant production". Thus, synseed production was previously limited to those plants in which somatic embryogenesis had been reported. However, many plant species remain recalcitrant to somatic embryogenesis. However, Bapat et al. (1987) proposed that synseeds could be produced from *in vitro* derived propagules other than somatic embryos, especially in non-embryogenic species; in *Morus indica*, for example, they proposed the use of encapsulated axillary buds. Thus, a synseed is referred to as artificially encapsulated somatic embryo, shoot bud or any other meristematic tissue that can be used as functional mimic seed for sowing, possesses the ability to convert into a plant under *in vitro* or *ex vitro* conditions, and can be stored (Ara et al., 2000; Capuano et al., 1998). This definition extends the concept of the synthetic seed from its bonds to somatic embryogenesis and links the term to its use (storage, sowing) and product (plantlet). In response to this shortcoming, the possibility of using non-embryogenic vegetative propagules such as shoot tips, nodal segments/axillary buds, protocorm like bodies (PLBs), organogenic or embryogenic callus has been explored as a suitable alternative to somatic embryos (Ahmad and Anis, 2010; Ara et al., 2000; Danso and Ford-Llyod, 2003; Faisal and Anis, 2007; Nhut et al., 2005; Ozudogru et al., 2011; Rai et al., 2008b; Sharma et al., 2009a,b; West and Preece, 2009). To complete this definition, it should be emphasized that the propagule must be able to grow into a plantlet after sowing (Piccioni, 1997).

Even though *in vitro*-derived propagules were used in most synseed studies for encapsulation, it is also possible to encapsulate propagules excised directly from *in vivo* cultivated mature plants. For example, Pattnaik et al. (1995) successfully encapsulated the dormant vegetative buds of an *in vivo*-grown three-year old mature mulberry tree. More recently, Banerjee et al. (2012) produced synseed containing young sprouted vegetative microshoots together with a small basal rhizome portion excised from *in vivo*-grown rhizomes of *Curcuma amada* which were stored in lightly packed polythene packets.

Over the past two decades, extensive progress has been made in synseed technology. Rai et al. (2009) presented a brief overview on synseed technology development in fruit crops only while Ara et al. (2000) and Saiprasad (2001) described the applications, prospects and limitations of sysnseed technology, but both those reviews are either incomplete, or outdated. The present review provides an up-to-date, elaborate and refreshing perspective on synseed technology covering as wide a range of plant species as possible.

Synseed technology is highly promising for the conservation and mass clonal propagation (Singh et al., 2006) of valuable rare hybrids, elite genotypes, sterile unstable genotypes and genetically engineered plants for which seeds are either not available or that require a mycorrhizal-fungal association for their germination as in the case of orchids. Recently, encapsulation technology has attracted the interest of researchers for germplasm delivery and for various analytical studies (Ara et al., 2000). The possible applications of synseed are summarized in Fig. 1.

2. Types of synseed

Since the formulation of the concept of synseed by Murashige (1977), a number of studies have been undertaken in this area of plant biotechnology. The basic hindrance to synseed technology was the lack of a natural endosperm and protective coatings in somatic embryos that made them inconvenient to store and handle (Redenbaugh et al., 1993). Furthermore, the absence of a quiescent resting phase and the inability of undergoing dehydration limited the utility of somatic embryos as a source of synseed production. Thus, the primary effort in synseed technology was to treat somatic embryos in such a way that they mimicked zygotic embryos during storage and other applications. This was the first major step in the success of synseed technology (Ara et al., 2000). Synseed technology has been extended by several research groups for a variety of plant species including cereals, fruits, vegetables, medicinal plants, forest trees, orchids and other ornamentals (Ara et al., 1999; Germanà et al., 1998; Ipekci and Gozukirmizi, 2003; Janeiro et al., 1997; Rai et al., 2008a,b; Utomo et al., 2008). Based on the literature available to date, synseeds can be separated into two categories:

2.1. Encapsulated desiccated

Coated desiccated embryos represent an ideal form of synseed (Pond and Cameron, 2003) for which somatic embryos are first hardened to withstand desiccation before encapsulation. This induces quiescence in the embryos and provides more handling flexibility in large-scale production systems. Thus, the ability of somatic embryos to withstand drying to low moisture content is an important factor for storage and plays a critical role in the developmental transition between maturation and conversion. Such types of synseeds can only be produced in those plants whose somatic embryos are desiccation-tolerant.

Desiccation can be achieved either slowly over a period of one or two weeks sequentially using chambers of decreasing relative humidity, or rapidly by unsealing the Petri dishes and leaving them overnight to dry (Ara et al., 2000). The drying rate is one of the critical factors for the efficient survival of somatic embryos. If the embryos are immature, slow drying over one week is optimal, but if there is large number of fully mature embryos, rapid drying in a laminar flow bench is preferable (Senaratna et al., 1990).

Desiccation tolerance can also be induced with maturation medium with high osmotic potential induced by either increased levels of permeating osmoticants (e.g., sucrose, mannitol), non-permeating osmoticants (e.g., polyethylene glycol or PEG) or high gel strength media (to limit water availability). While working with ginger synseeds, Sundararaj et al. (2010) found that sucrose-dehydration was more effective than air-dehydration in terms of re-growth ability by providing required nutrients; moreover, rapid moisture loss during air dehydration resulted in poor conversion frequency. For sucrose-dehydration, Sundararaj et al. (2010) transferred the synseeds to liquid nutrient medium containing various concentrations of sucrose for 16 h and kept them in an incubator-shaker for 16 h at 25 ± 2 °C. Synseeds dehydrated resulted in 86% conversion whereas higher concentrations (0.50 M and 0.75 M) resulted in no conversion.

Other sub-lethal stresses such as low temperature and nutrient deprivation also have a similar effect on desiccation tolerance (Pond and Cameron, 2003). Properly pretreated embryos remain viable when they are rapidly desiccated to less than 10–15% moisture content. Pretreatment with abscisic acid (ABA) also improves the conversion of somatic embryos both in desiccated and hydrated systems (Nieves et al., 2001; Pond and Cameron, 2003). Nieves et al. (2001) reported the effect of ABA and jasmonic acid (JA) on partial desiccation of encapsulated sugarcane somatic embryos. Before encapsulation, embryogenic callus with somatic embryos were placed on MS medium supplemented with 3.8 μM ABA and/or 4.7 μM JA, as described by Tapia et al. (1999),

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