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Research review paper

The encapsulation technology in fruit plants—A review

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ABSTRACT

Encapsulation technology is an exciting and rapidly growing area of biotechnological research. This has drawn tremendous attention in recent years because of its wide use in conservation and delivery of tissue cultured plants of commercial and economic importance. Production of synthetic seeds by encapsulating somatic embryos, shoot buds or any other meristmatic tissue helps in minimizing the cost of micropropagated plantlets for commercialization and final delivery. In most of fruit crops, seed propagation has not been successful because of heterozygosity of seeds, minute seed size, presence of reduced endosperm, low germination rate, and also some are having seedless varieties. Many species have desiccation-sensitive intermediate or recalcitrant seeds and can be stored for only a few weeks or months. Under these circumstances, increasing interest has been shown recently to use encapsulation technology for propagation and conservation. Many fruit plants are studied worldwide for breeding, genetic engineering, propagation, and pharmaceutical purposes. In this context, synthetic seeds would be more applicable in exchange of elite and axenic plant material between laboratories and extension centers due to small bead size and ease in handling. Due to these advantages, interest in using encapsulation technology has continuously been increasing in several fruit plant species. The purpose of this review is to focus upon current information on development of synthetic seeds in several fruit crops.

Contents

1. Introduction

Fruit crops are being cultivated since olden times as fruits are very important dietary components. Fruits have essential roles in the maintenance of life due to their high nutritive value and are important

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in running the economy of many peoples due to their commercial value. However, several biotic (diseases, insects, pests etc.) and abiotic (salt, drought, heat, cold etc.) stresses are constant factors that limit the production of fruit plants. As many of the fruit plants are large trees and have a prolonged juvenile phase, their improvement through traditional breeding methods is not possible (Jaiswal, 2003). In most of the fruit crops, seed propagation has also not been successful. This may be due to heterozygosity of seeds, minute seed size, presence of reduced endosperm, low germination rate, and also because some are having seedless varieties (Saiprasad, 2001). Conventional propagation methods such as grafting, air layering, stooling etc. for improving many fruit trees already exist but extended juvenility has made these techniques time consuming and cumbersome. Plant tissue culture offers an effective solution of such problems of propagation of fruit crops. For improvement of fruit crops through several biotechnological approaches, highly efficient regeneration is a prerequisite (Litz and Jaiswal, 1991).

Many fruit species particularly tropical and subtropical fruit plants have characteristics that make it difficult to conserve them by using traditional methods. Many species have desiccation-sensitive intermediate (i.e. papaya, banana, citrus etc.) or recalcitrant seeds (i.e. mango, avocado, litchi, jackfruits etc.) and can be stored only for from weeks to few months. This is mainly because of their high metabolism and high incidence of fungal infection. In addition, collections of germplasm from field gene banks are exposed to natural disasters, attacks by pests and pathogens and labour costs are very high. Distribution and exchange from field gene banks are difficult because of vegetative nature of the material and greater risk of disease transfer (Chaudhury and Malik, 2003). To circumvent these problems, increasing interest has been shown recently to use encapsulation technology for conservation and germplasm exchange of these species. This write up presents a brief overview of current status of development of synthetic seeds in several fruit crops and their role in conservation and germplasm exchange.

2. Synthetic seed: concept, process and application

The concept of synthetic seed was given by Murashige (1977), but first report on the development of synthetic seeds was published by Kitto and Janick (1982). They reported the production of desiccated synthetic seeds by coating a mixture of carrot somatic embryo in a water-soluble resin, polyoxyethylene glycol (Polyox). Later, Redenbaugh et al. (1984) were successful in producing synthetic seeds for alfalfa by encapsulating somatic embryos with alginate hydrogel. Since then several research groups have been working on synthetic seeds with different plant species including cereals, fruits, vegetables, ornamentals, medicinal plants, forest trees and orchids (Bapat et al., 1987; Bapat and Rao, 1988; Mathur et al., 1989; Ganapathi et al., 1992; Corrie and Tandon, 1993; Sharma et al., 1994; Maruyama et al., 1997; Sarkar and Naik, 1998; Ara et al., 1999; Mandal et al., 2000; Sicurani et al., 2001; Rout et al., 2001; Nyende et al., 2003; Chand and Singh, 2004; Singh et al., 2006a,b; Naik and Chand, 2006; Micheli et al., 2007; Faisal and Anis, 2007; Rai et al., 2008a,b; Singh et al., 2009).

A synthetic seed or artificial seed is referred to as artificially encapsulated somatic embryo, shoot bud or any other meristmatic tissue that can be used as functionally mimic seed for sowing and possesses the ability to convert into a plant under *in vitro* or *ex vitro* conditions and that can retain this potential even after storage (Capuano et al., 1998; Ara et al., 2000). Earlier, the concept of synthetic seeds was based only on the encapsulation of somatic embryos that could be handled like a real seed for transport, storage and sowing, but, in recent years, the encapsulation of non-embryogenic vegetative propagules like apical shoot buds, axillary buds, nodal segments, etc. have also been employed as a suitable alternative to somatic embryos (Sarkar and Naik, 1998; Standardi and Piccioni, 1998; Ara et al., 2000; Danso and Ford-Lloyd, 2003; Bapat and Mhatre, 2005; Rai et al., 2008b). The main advantage of these non-embryogenic vegetative propagules would be in those crops where either somatic embryogenesis is not well established or do not produce uniform quality embryos. In such cases synthetic seed system may be useful for propagation and delivery of tissue cultured plants (Rao et al., 1998).

Based on technology established, there are two types of synthetic seeds: hydrated and desiccated. Although, the most studied method involves the encapsulation of propagules in hydrogel for synthetic seed production (Redenbaugh and Walker, 1990). A number of coating agents such as sodium alginate, potassium alginate, carrageenan, sodium alginate with gelatin, sodium pectate, carboxymethyl cellulose etc. are used for encapsulation and among these substances sodium alginate has been extensively used (Redenbaugh et al., 1987; Rao et al., 1998; Ara et al., 2000). Due to absence of a nutritive tissue like the endosperm of the natural seed, synthetic seeds have low conversion ability in some cases (Arun Kumar et al., 2005). Addition of nutrients, carbon sources, growth regulators and antimicrobial agents such as antibiotics, fungicides etc. in the gel matrix which apparently served as a synthetic endosperm, facilitated growth and survival of encapsulated propagules (Redenbaugh et al., 1987; Gray, 1990; Bapat and Mhatre, 2005). Such additives should be non-toxic to propagules and allow the development of plants without any variation (Redenbaugh and Ruzin, 1989; Bapat and Mhatre, 2005). Hindrance of the gel capsule for the emergence of the root and shoot from encapsulated propagule is another mechanical problem in encapsulation technology, although, adopting the self-breaking alginate gel beads technology could overcome this shortcoming (Onishi et al., 1994). Calcium alginate capsule pretreated with potassium nitrate becomes soften and allow the easily emergence of shoot and root from alginate beads (Onishi et al., 1994). Application of potassium nitrate in the breaking of alginate capsule has also been reported in a few plant species (Guerra et al., 2001; Arun Kumar et al., 2005).

To produce hydrated synthetic seeds, the propagules (somatic embryos, shoot buds, nodal segments etc.) are carefully isolated from *in vitro* cultures and mixed with encapsulation mixture [sodium alginate (0.5–5.0% w/v) prepared either in double distilled water or liquid nutrient medium] and dropped into a complexing agent such as calcium chloride or calcium nitrate solution (30–150 mM). After 30–40 min incubation in calcium chloride solution, alginate beads become hard. An ion-exchange process takes place during this period resulting in the replacement of sodium ions by calcium ions forming calcium alginate (Redenbaugh and Walker, 1990; Ara et al., 2000). Hardening of calcium alginate bead is affected by the concentration of sodium alginate and calcium chloride and it may vary with different propagules as well as with different plant species. After washing with sterile double distilled water such encapsulated propagules are cultured on nutrient medium or different substrates like wet filter paper, cotton or soilrites with medium or double distilled water for plantlet conversion.

2.1. Advantages of synthetic seeds

Encapsulation technology is an exciting and rapidly growing area of seed biotechnological research. It has considerable impact on conservation and delivery of tissue cultured plants in a more economical and convenient way (Rao et al., 1998). The scope of synthetic seeds is presented in Fig. 1. It is an excellent technique for propagation of rare hybrids, elite genotypes, genetically engineered plant, and rare and endangered plants for which the seeds are either very expensive or are not available (Mandal et al., 2000). As the production costs for hybrid seeds (in vegetable, forage, cereals or commercially important plants) or other conventional propagation methods are very high, synthetic seeds should offer a low cost alternative (Rao et al., 1998). Some other potential advantages of encapsulation technology include ease in handling (due to small size of capsule), genetic uniformity of plants and direct delivery to the field