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Original Article

Synthetic seeds: Production and applications in medicinal and aromatic plants

Hari Kesh

College of Horticulture & Forestry, Central Agricultural University (Imphal), Pasighat-791102

**Corresponding Author: harikeshkaul55v2@gmail.com*

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1. INTRODUCTION

Medicinal and aromatic plants (MAPs) constitute a big group of plants used since ancient time for the treatment of many diseases like cancer, cardiovascular, depression, anxiety, Alzheimer's Parkinson's and neurological diseases and for therapeutic, flavouring and fragrance in pharmaceutical and cosmetic industries (Goncalves et al., 2020). Medicinal plants are an important source of bioactive compounds that can be used drugs either directly or indirectly for the medical purposes while, aromatic plants are rich source of essential oils, alkaloids and phenolic compounds that are used for their aroma and flavour (Manousi et al., 2019). Up to 80 % people in developing countries are totally dependent on herbal drugs for their primary healthcare further, with the increasing demand for herbal drugs and secondary metabolites of medicinal plants the use of medicinal plants is growing rapidly throughout the world (Cole et al., 2007). It has been reported that the flowering plants which are used for medicinal purpose, many of them are threatened with extinction from habitat destruction and overexploitation. A number of conservation strategies including both in-situ and ex-situ have been developed (Chen et al., 2016). Synthetic seed technology is considered as a boon for the protection, conservation and germplasm exchange of many medicinal and aromatic plants. Synthetic seeds are encapsulated somatic embryos, apical shoot buds, nodal explants or any other meristematic tissue that can be used as a seed for sowing purpose and has the potential to develop into a complete plant (Sharma et al. 2013). Synthetic seed production facilitates the ex-situ conservation of rare and endangered plant species, plants producing minimum number of seeds or producing recalcitrant seeds, propagation of crops having low germination rate, propagation of seedless cultivars and multiplication of heterozygous genotypes. Many medicinal and aromatic plant species produce desiccation sensitive seeds and can be stored for only few weeks to months. The desiccation sensitive seeds remain metabolically active and cannot tolerate moisture loss. Under such conditions, synthetic seed technique is a potential approach for long-term storage of the germplasm of desiccation sensitive species. Apart from the conservation and storage of genetic material, it allows easy handling and transporting, virus free germplasm, minimum cost of production and genetically identical plants. However, a number of factors like selection of explant material, encapsulating agent and matrix significantly affects the success of synthetic seed production and their storage (Gantait et al., 2015). In this article, we discussed the challenges, opportunities and applications of synthetic seed technology in medicinal and aromatic plants.

2. Selection of Plant Materials

a) Somatic Embryo

Somatic embryos (SEs) have a great potential for synthetic seed production technology because of their biplor nature. SEs originates from somatic tissues and possess both radicle and plumule axis compared to non-embryogenic tissues. Theoretically, somatic cells start embryo development either through gaining embryogenic cell differentiation via induction or losing somatic cell differentiation via reversion (Yucesan, 2019). It means, the somatic cells are not naturally embryogenic but they can become so in response to internal (epigenetic) or external (auxin concentrations) factors under certain circumstances. The plants species which have the ability of producing somatic embryos maintain their regeneration potential for a long period of time by passing interceding callus stage. Therefore, SEs can be produced in bulk, making them most suitable propagule for encapsulation. The SEs also undergoes maturation and accumulation of carbohydrate, storage lipids, proteins, and plant hormones like their zygotic counterparts (Winkelmann 2016). SEs have been produced successfully in several MAPs like *Dioscorea floribunda*, *D. deltoidei*, *Artemisia vulgaris*, *Anethum graveolens*, *Rotula aquatica*, *Arnebia euchroma*, *Hemidesmus indicus*, *Swertia chirayita*, *Quercus robur*, *Rotula aquatic*, *Pinus Clitoria ternatea*, *Rhinacanthus nasutus*, *Catharanthus roseus* etc. (Saxena et al., 2019). Despite the remarkable advantages of SEs over the other plant propagules, there are some hurdles in producing synthetic seeds, causing a varying degree of success with SEs in MAPs. The major hurdles include asynchronous and late development of embryonic poles, regeneration potential of encapsulated SEs and poor germination rate (Castellanos et al. 2004). Furthermore, genotypic variations were also observed for embryogenic ability in MAPs.

b) Protocorm-Like Bodies

In Orchids, the embryos develop into a unique structure called as Protocorm. The main function of which is the formation of shoot apices. Protocorms are produced by embryos while protocorm like bodies (PLBs) are produced form tissue explants particularly in vitro conditions. The PLBs are bipolar structure and produce root and shoot upon development (Gnasekaran et al. 2016). PLBs looks like somatic embryos and provide an efficient way of propagation in orchids. The synthetic seed production using PLBs are reported in *Dendrobium nobile* and *Vanda coerulea* (Mohanty et al. 2013). Although, generative response of PLBs appears to take much longer tome compared to direct shoot regeneration, the propagation efficiency appears to be are more (Thung et al., 2022). Synthetic seed production using PLBs heve been documented in *Flickingeria nodosa*, *Aranda* × *Vanda* and hybrid *Cymbidium* Twilight Moon 'Day Light'.

c) Nodal Segment or Microcuttings

Nodal segments (NS) with axillary buds also called microcuttings are shoot segments having one or more buds. The NS are most used non-embryonic explants used for synthetic seed production due to ease in their production, ability to retain viability and high proliferation rate after a longer period of storage, minimum physiological variation and easyness in transport (Benelli 2016). However, one of the major limitations of NS is the inability of shoots to develop roots particularly in recalcitrant and woody plants. NS have been successfully used for synthetic seed production in a number of plants like *Khaya senegalensis*, *Rauvolfia serpentina*, *Stevia rebaudiana*, *Centella as iatica*, *Salvia splendens*, *Balanites aegyptiaca* and *Rosa damascena f. trigintipetala* (Reviewed in Qahtan et al., 2019).

d) Shoot Tips

The shoot tips (STs) with actively dividing meristematic cells are the space and cost-effective propagules used second most after nodal segment in synthetic seed production. The apical meristem in STs diminishes the chances of transfer of plant pathogen specifically viruses to the newly produced progenies, thus making them an efficient explant source for encapsulation. Further, due to high mitotic activity in meristematic cells, they responded greatly to plant growth regulators producing multiple number of shoots. STs were used effectively in synthetic seed production many aromatic and medicinal crops like *Picrorhiza kurrooa*, *Stevia rebaudiana*, *Mentha arvensis*, *Ceropegia spiralis*, *C. pusilla*, *Terminalia arjuna*, *Bacopa monnieri* and *Withania somnifera* etc. (Ghosh and Haque, 2019).

3. Selection of the Encapsulation Matrix

The encapsulation material is a critical factor to produce uniform synseeds. The encapsulating material act as a coating around the explant which involves mineral elements, source of carbohydrate, growth regulators, etc. in water or standard nutrient media (MS and B5 etc.) and acts as an artificial endosperm. This coat provides the essential nutrients for proper growth and shields the explants during storage and handling. However, the material should be consistent that allow easy seed handling without breakage and weak enough to allow the bud to break the capsule upon regrowth (Redenbaugh et al. 1986). During the initial years, much of the research was carried out for the selection of suitable encapsulation agents like sodium alginate, gelrite, agarose, potassium alginate, sodium pectate, guar gum, carrageenan etc. Among all these encapsulation agents, sodium alginate was used commonly due to its useful thickness, rapid gelation, low cost, as well as non-toxic to plants (Gantait et al. 2015). Furthermore, the addition of nutrients and growth regulators to the encapsulation matrix is also an important as it increases the reliability of germination and the viability of the synseeds. The optimum concentration of sodium alginate reported to be 3% with 100 mM CaCl2 for synthetic seed production in plant species including *Manihot esculenta*, *Erythrina variegata* and *Salix tetrasperma*. However, sodium alginate 2% with CaCl2 of 50 mM was found to produce high quality beads in *Artemisia vulgaris* by encapsulating nodal segments (Sujatha and Kumari 2008).

4. Applications of Synseeds

a) Propagation

Synseeds could be used as an efficient practice for mass propagation and multiplication of rare and endangered plants, seedless plants, medicinal plants, and commercially important plants (Gantait et al. 2015). These seeds can be effectively grown in vitro, on culture medium and on planting substrate like perlite, vermiculite, soil, soilrite, sand, vermicompost for regeneration into complete plantlets. In general, the regrowth ability of encapsulated explants to complete plantlets is more effective on nutrient-rich medium than on nutrient-deficient (Sharma et al. 2013). Baskaran et al. (2017) obtained 91% shoot regeneration of encapsulated shoot tips of *Urginea altissima* on semi-solid MS medium containing 10 µM mT and 2 µM NAA. Likewise, Dhir and Shekhawat (2013) reported maximum conversion response of synseeds into plantlets of *Ceropegia bulbosa* on medium supplemented with 8.88 µM BA.

b) Short- and Medium-Term Conservation

Synthetic seed technology provides greater options for short- and medium-term conservation of different plant species. For commercial utilization of this technology like during transportation and conservation, it is primarily necessary to have flexible storage options and effective regeneration even after storage. Most found suitable temperature for short- and medium-term storage of synthetic seed varied depends upon the plant/tree species (Sharma et al. 2015). In general, low temperature at 4 C is found suitable in most of the earlier studies. In beads of *Rauvolfia serpentina* stored at different temperatures (20, 12, and 4 C) indicated that 4 C to be the most suitable with 68.5-100% regrowth up to 14 weeks (Ray and Bhattacharya, 2008).

c) Reintroduction

Synseed technology can be used for the reintroduction of rare, threatened, and endangered plant species. There are some examples of endangered plant species that are multiplied and reintroduced in their natural habitat like *Cochlearia bavarica*, *Castilleja levisecta*, and *Renanthera imschootiana*. This technology makes the reintroduction process faster as the synseeds takes less time for preparation and easy to transport (Saxena et al., 2019).

d) Direct sowing

Synseed can be sown directly in ex vitro plating media like sand, vermicompost and perlite because these seeds do not need to be acclimatization, that is essentially required for micro propagated plants (Mandal et al. 2000). Direct sowing has been observed successful in many plant species like *Dalbergia sissoo*, *Phyllanthus amarus* and *Erythrina variegata*. The major problem in direct sowing of synseeds is the contamination by microbes. This problem however, can be rectified by using fungicides like carbendazim or bavistin and benomyl @ 50-100 mg L^{-1} in the encapsulation matrix (Saxena et al., 2019).

5. Future Perspectives

Synseed technology offers excellent scope for the propagation, conservation and germplasm exchange of different medicinal, aromatic and forest crops. However, commercialization of this technology cannot be amplified unless the major limitations are rectified. For example, large-scale production of synseeds with high regeneration ability, automation of encapsulation and regeneration methods, use of non-embryogenic propagules to produce synseeds, best nursery medium, storage temperature, and its duration requires further research and investigation. Consideration of efficient gelling agent, constituents of gel matrix and formulations of artificial endosperm also worth further research for practical application of this technology.

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