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ORIGINAL RESEARCH

Encapsulation of Protocorm Like Bodies and *in vitro* regeneration of *Asparagus racemosus* Willd.

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ABSTRACT
Various concentrations of sodium alginate and calcium chloride were tested in order to optimize the shape, and texture of synthetic seeds developed through the encapsulation of Protocorm Like Bodies (PLBs) of *Asparagus racemosus*. Sodium alginate (2.5%) and $CaCl_2.2H_2O$ (100 mM) solutions were found to be the optimal concentration for production of firm, transparent and uniform synthetic seeds. The beads showed 94.9% germination on MS basal medium within three weeks. The germinated seedlings formed complete plantlet having well developed root-shoot system. The developed protocol for synthetic seed production through PLBs can be used for conservation of *A. racemosus*.

KEY WORDS: *Asparagus racemosus, Protocorm like bodies, Synthetic seed, In vitro regeneration*

INTRODUCTION

Asparagus racemosus Willd. (Asparagaceae, Fig. 1 A), an important medicinal plant of tropical and subtropical India, is a potent phytoestrogen which is used as traditional medicine to treat various ailments such as menopausal symptoms, diarrhea, dyspepsia, inflammations, cough, bronchitis, hyperacidity, neurodegenerative disorders etc. (Alok *et al.,* 2013). The major active constituents of *A. racemosus* are steroidal saponins, which are present in the roots. Leaves contain rutin, diosgenin and a flavonoid glycoside identified as quercetin-3-glucuronide. Flowers contain quercetin hyperoside and rutin. Fruits contain glycosides of quercetin, rutin and hyperoside and steroidal saponins (Mandal *et al*., 2006). Due to destructive harvesting, habitat destruction, and deforestation, the plant is now considered threatened in its natural habitat. Efficient conservation practices for *A. racemosus* can be obtained by a combination of various biotechnological methods. The development of an efficient micro-propagation protocol will play a significant role in conservation of *A. racemosus* in its natural habitat.

Synthetic seed production is a well documented applied technology that capitalizes on the capacity for rapid plant multiplication via somatic embryogenesis (Castillo *et al.,* 1998). It is a rapidly growing area of biotechnology as deals with *in vitro* conservation and storage of rare, endangered and desirable genotypes along with its easy handling and transportation (Germana *et al.,* 2011). However, there are no reports available at present on the development of synthetic seed for *A. racemosus*. Hence, the present study was undertaken to standardize various concentrations of sodium alginate and calcium chloride solutions in order to optimize the shape and texture of synthetic seeds produced through encapsulation of Protocorm Like Bodies (PLBs) of *A. racemosus*. Encapsulated PLBs were also successfully

germinated *in vitro*.

RESULTS AND DISCUSSION

The attempts to encapsulate PLBs have been done on many plant species to produce synthetic seeds (Mohanraj *et al*., 2009; Sarmah *et al*., 2010; Nagananda *et al.,* 2011). In the present investigation, artificial seeds were produced from encapsulated PLBs obtained from the nodal explants of *A. racemosus*. Observations were made after beads formation in $CaCl_2.2H_2O$. It was found that encapsulated PLBs showed different degrees of success based on bead formation (Table 1). The beads which were formed at higher concentration of sodium alginate solution (3.5%) and dipped in 100 mM $CaCl_2.2H_2O$ solution were rigid, firm, clear and isodiametric and suitable for handling. On the other hand, beads that were formed in 3.5% sodium alginate solution and dipped in 50/75 mM $CaCl_2.2H_2O$ solution were of uniform size, isodiametric, solid and quite rigid. These beads had a short tail on the surface. The beads, which were formed by using 2.5% sodium alginate solution and 100 mM $CaCl_2.2H_2O$ solution as a complexation process were of uniform size, isodiametric, clear, solid and were ideal for the present study (Fig. 1 B). On the other hand, beads formed in 2.5% sodium alginate and hardened in 75 mM $CaCl_2$ solution had solid texture with clusters and those hardened in 50 mM $CaCl_2.2H_2O$ solution gave malformed beads, which were too soft to handle.

At lower concentration of sodium alginate (1.5%) and higher concentration of $CaCl_2$. $2H_2O$ 75–100 mM bead formation was very poor, *i.e.* PLBs did not get coated. Lower concentration of the calcium chloride did not support the proper ion exchange for firm bead formation which agrees with the findings of Sarmah *et al*. (2010).

In the present study, sodium alginate (2.5%) and $CaCl_2.2H_2O$ (100 mM) solutions were found to be the optimal concentration for ideal bead formation. The beads showed 94.9% germination in MS basal medium within three weeks (Fig. 1 C). The germinated seedlings formed complete plantlet having well developed root-shoot system (Fig. 1 D). In conclusion, this study developed highly effective technique for synthetic seed production through PLBs for conservation of *A. racemosus*.

Table 1: Effect of different concentrations of sodium alginate and $CaCl_2.2H_2O$ on formation of encapsulated beads

Conc. of sodium alginate (%)	Conc. of $CaCl_2.2H_2O$ (mM)	Nature of bead formation	Remarks
1.5	50	+	Fail to coat PLBs
1.5	75	++	Too soft and very fragile
1.5	100	++	Poor bead formation
2.5	50	+	Malformed beads, very fragile and soft to handle
2.5	75	++	Solid texture and formed clusters
2.5	100	+++++	Clear, firm, round and uniform size
3.5	50	++++	Uniform size and isodiametric
3.5	75	++++	Uniform size, isodiametric and quite rigid
3.5	100	++++	Rigid, firm, clear and isodiametric

MATERIALS AND METHODS

PLBs obtained from nodal explants of *A. racemosus* were immersed in 1.5, 2.5, and 3.5 sodium alginate solution containing half MS medium with 2% (w/v) sucrose. PLBs, mixed in sodium alginate solution, were dropped individually with a pipette into 50, 75 and 100 mM $CaCl_2.2H_2O$ solution prepared in half Murashige and Skoog (1962) medium with 2% (w/v) sucrose. The synthetic encapsulated beads were washed with sterile distilled water and collected by filtration. Finally, the encapsulated PLBs were cultured on MS medium supplemented with 30 g/L of sucrose and 7 g/L of agar-agar.

All the cultures were incubated in the growth room having the light intensity of 1200 lux from fluorescent tubes and incandescent bulbs at the temperature of 26±2 ^{O}C. A photoperiod of 16 hours was maintained with the help of a timer (Yamasa, Japan).

Figure 1: (A) *Asparagus racemosus,* (B) encapsulated PLBs, (C) *in vitro* germination of encapsulated PLBs and (D) complete plantlet

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