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Ascorbic acid as a growth adjuvant in encapsulated protocorm-like bodies of *Rhynchostylis retusa* Bl. (Orchidaceae)

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ABSTRACT

In the present study, effect of ascorbic acid, a known growth adjuvant on encapsulated protocorm-like bodies (PLBs) of *Rhynchostylis retusa* Bl. was investigated. PLBs were encapsulated in calcium alginate (3.5% sodium alginate and 100 mM calcium chloride) prepared in Mitra *et al.* (1976) basal medium and supplemented with different concentration of ascorbic acid (5, 10, 15 and 20 mM). The encapsulated PLBs were stored at 25°C. Their germination response and germination potential were evaluated after every 4 weeks on basal media. Control set of encapsulated PLBs, failed to germinate after 32 weeks. However, PLBs with 15 mM ascorbic acid in the encapsulated matrix showed the best response; nearly 90% germinated even after 32 weeks of storage. The survival and germination frequency was directly proportional to the level of ascorbic acid in the alginate mix up to 15 mM level but declined on further increase. Differentiation of PLBs into plantlet was better in synthetic seeds containing lower concentration of ascorbic acid (5 mM) as compared to higher levels (15, 20 mM) whereas multiplication of secondary PLBs was more pronounced at higher levels. Chlorophyll content was inversely proportional to the level of ascorbic acid in the nutrient mix; lush-green PLBs were observed at low concentration of ascorbic acid (5 mM). This study highlights the potential of ascorbic acid as an aid to growth and survival of encapsulated PLBs upon storage.

KEY WORDS: Ascorbic acid, encapsulated protocorm-like bodies, Orchidaceae, *Rhynchostylis retusa*

INTRODUCTION

Orchidaceae constitute one of the largest families of angiosperms. Orchids are most valued as cut flowers and potted plants (Britt, 2000). Orchids are also acclaimed for their medicinal properties since Vedic times because of their rich phytochemical constituents mainly alkaloids, flavonoids, and glycosides (Jalal *et al.,* 2008). *Rhynchostylis retusa* Bl., an epiphytic fox-tail orchid is well known for its ornamental value owing to its floriferous nature (Vij *et al.*, 1984). It is also reported to help in the treatment of dysentery, tuberculosis, epilepsy, menstrual disorders, fever, gout, asthma, rheumatism, and malarial fever (Radhika and Murthy, 2013). Due to its immense importance, it has been indiscriminately collected from the wild. Anthropogenic pressures and deforestation have further pushed the species on the verge of being threatened (Yagya and Fischer, 2011). Hence, there is a growing need to conserve this species. Encapsulated seed production has proven to be a better approach than other conventional *in vitro* propagation methods as the seeds are easy to handle, store, and transport; however, with limitations of loss of viability upon long-term storage (Shawky, 2006). There are various reports highlighting the use of encapsulated seed production for propagation and conservation of threatened orchids (Pradhan *et al.*, 2014; Gantait and Sinniah, 2013; Mohanty *et al.*, 2013; Siew *et al.*, 2014).

It has been reported that addition of growth adjuvants can substantially increase the efficiency of synthetic seeds upon long-term storage. A well-established role of ascorbic acid as an antioxidant (Stasolla and Yeung, 1999) and enzyme cofactor (Foyer, 1993) has been reported in plants. Ascorbic acid acts as primary substrate for detoxifying hydrogen peroxide and neutralizes superoxide radicals and single oxygen. It also acts as a secondary antioxidant

for reductive recycling of α-tocopherol, a lipophilic antioxidant molecule (Noctor and Foyer, 1998). Hence, the present study was undertaken to investigate the role of ascorbic acid in conversion and growth of encapsulated protocorm-like bodies (PLBs) of *R. retusa* Bl.

MATERIALS AND METHODS

Plant Material

A commercially important orchid species, namely, *R. retusa* Bl. was selected for the present study. Green capsules (24WAP) were collected from naturally growing populations on *Mangifera indica* trees in Dehradun area (Uttaranchal, India). These were cultured asymbiotically following Kumar *et al.* (2002) to ensure regular supply of plant material. Synchronous PLBs to be used for encapsulation were obtained by clonal propagation using juvenile leaf explants (Vij *et al.*, 1984).

Encapsulation

Encapsulated PLBs were prepared by the complexation between sodium alginate (3.5 g/L) and calcium chloride (100 mM). The propagules were dispersed in sterile (autoclaved at 1.1 kg/cm^2, 121°C for 15 min) sodium alginate matrix prepared in Mitra *et al.* (1976; M) medium supplemented with different concentrations of filter sterilized ascorbic acid. The suspension was then added drop wise (each drop containing a propagule) to stirred calcium chloride solution. The resultant beads were complexed for 30 min and washed thoroughly with sterile distilled water and stored at 25°C.

Data Recording and Analysis

The germination potential was recorded periodically at 4 weeks interval up to 32 weeks by inoculating the encapsulated PLBs on fresh basal M medium. The data were collected in triplicate and represented as means (with 5% error bar).

RESULTS AND DISCUSSION

Studies were carried out to evaluate the efficacy of ascorbic acid in enhancing growth and survival upon long-term storage in encapsulated PLBs of *R. retusa*. Ascorbic acid (5, 10, 15, 20 mM) was supplemented in encapsulated mix to check its potential as a growth adjuvant.

Ascorbic acid in different concentrations was added to the matrix before encapsulation of PLBs. The germination potential was evaluated at a 4 weeks interval. In the control set of experiments, the encapsulated PLBs failed to germinate after 32 weeks of storage at 25°C. The presence of ascorbic acid in the encapsulation matrix; however, facilitated 60-90% survival (Graph 1) after the same period. The survival percentage increased with increased level of ascorbic acid; up to 15 mM which showed the best response (90%) (Graph 1). This can be corroborated by a previous study by Sembi *et al.* (2014) in which 15 mM of exogenous ascorbic acid-favored high germination response in encapsulated seeds.

Chlorophyll content in plantlets obtained from the encapsulated PLBs was inversely proportional to the level of ascorbic acid in the nutrient mix as evident from visual observations; lush-green leaves were observed in ascorbic acid (5 mM) and the chlorophyll content reduced with increase in ascorbic acid concentration (Figure 1a-c). Conversion of PLBs into plantlets was better in encapsulated PLBs containing lower concentration (5, 10 mM) of ascorbic

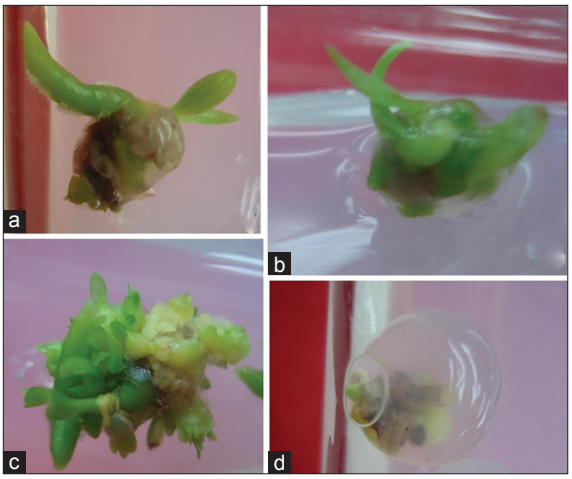
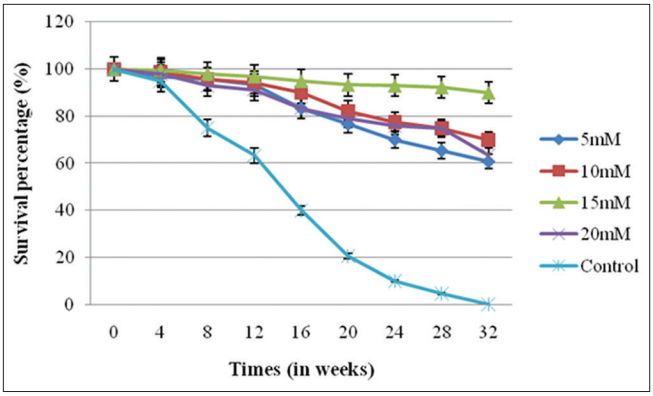


Figure 1: Morphogenetic response of encapsulated protocorm-like bodies (PLBs) of *Rhynchostylis retusa* under the effect of ascorbic acid (5, 10, 15, and 20 mM): (a) Lush-green leaves with high chlorophyll content (5 mM); (b) PLBs conversion (10 mM); (c) excessive somatic embryogenesis and low chlorophyll content (15 mM); (d) growth inhibition (20 mM)



Graph 1: Survival percentage of encapsulated protocorm-like bodies in *Rhynchostylis retusa* under the effect of ascorbic acid (5, 10, 15, and 20 mM) up to 32 weeks

Table 1: Different parameters observed of encapsulated PLBs in *Rhynchostylis retusa* under the effect of ascorbic acid (5, 10, 15, and 20 mM)

Parameters	Control	5 mM	10 mM	15 mM	20 mM
Differentiation of PLBs into plantlets	+	+++	++	+	+
Somatic embryogenesis	+	-	+	+++	+
Chlorophyll	+	+++	++	+	+
Survival rate after 32 weeks	-	+	++	+++	+

PLB: Protocorm-like bodies

acid (Figure 1a and b). Profuse somatic embryogenesis was observed at 15 mM (Figure 1c) while higher concentrations (20 mM) proved inhibitory (Figure 1d). Lower concentrations (5 mM) have been reported to be more suited for chlorophyll production and early differentiation of plantlets (Sembi *et al.*, 2014). Beneficial effects of ascorbic acid in photosynthesis have been highlighted by Miyake and Asada (1992) and Smirnoff (1996).

Ascorbic acid plays major role in inhibition of oxidation process (Babbar *et al.,* 2010) in addition it is showed by Ko *et al.* (2009) that it prevent browning of explants and increase the number of shoots. The use of activated charcoal and ascorbic acid in control of browning has also been reported by Abdelwahd *et al.* (2008), Nisyawati and Kariyana (2013), Zhou *et al.* (2010). In another study by Richard *et al.* (1988), enhanced organogenesis was reported in tobacco callus when ascorbic acid (0.4-0.8 mM) was added to the medium. Stasolla and Yeung (2001) also reported enhanced conversion into plantlet of white spruce somatic embryos under the effect of exogenous ascorbic acid (0.1 mM). Ascorbic acid at the concentration (0-30 mM) has been showed by Bybordi (2012) to play major role in inhibiting salt stress.

Our results and those of related studies suggest that ascorbic acid can effectively increase in germination potential, percentage growth, and differentiation on storage in encapsulated PLBs of *R. retusa*.

CONCLUSIONS

The present study highlights that exogenous ascorbic acid could play a pivotal role in improving germination, viability on storage, growth and differentiation of encapsulated PLBs and could serve as an aid to germplasm storage, multiplication, and conservation of *R. retusa* Bl as a growth adjuvant (Table 1 and Graph 1).

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