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...
MATERIALS AND METHODS

Plant Material

A commercially important orchid species, namely, *R. retusa* Bl. was selected for the present study. Green capsules (24 WAP) 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/cm², 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 acid.
Table 1: Different parameters observed of encapsulated PLBs in *Rhynchostylis retusa* under the effect of ascorbic acid (5, 10, 15, and 20 mM)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>5 mM</th>
<th>10 mM</th>
<th>15 mM</th>
<th>20 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differentiation of PLBs into plantlets</td>
<td>+</td>
<td>+++</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Somatic embryogenesis</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td></td>
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<tr>
<td>Chlorophyll</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Survival rate after 32 weeks</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

PLB: Protocorm-like bodies

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 Yung (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).

**ACKNOWLEDGMENTS**

The financial support by the Punjab State Council for Science and Technology, Chandigarh, India, is gratefully acknowledged.

REFERENCES


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