Callus Induction and Organogenesis from Explants of
Aconitum heterophyllum - Medicinal Plant

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Abstract: A protocol has been developed for in vitro shoot proliferation from callus cultures of Aconitum heterophyllum Wall. Callus initiation occurs from nodal segments on MS media fortified with NAA (0.5 mg L⁻¹) and BAP (0.25 mg L⁻¹). Callus was transferred on MS media supplemented with BAP (0.25 mg L⁻¹) for shoot proliferation. The best response for shoot proliferation was obtained on MS media + NAA (0.25 mg L⁻¹) + BAP (0.5 mg L⁻¹). The well-developed micro shoots were transferred to root induction media containing MS basal media + IAA (1.0 mg L⁻¹). The rooted plantlets were finally transferred to green house for hardening and field transfer.

Key words: Aconitum heterophyllum, in vitro propagation, nodal segments, explants

INTRODUCTION

Aconitum heterophyllum Wall. commonly known as ‘Atis’ or ‘Patis’ belongs to the family Ranunculaceae and is reputed for its medicinal and pharmaceutical values since long. It is a perennial herb, distributed over temperate parts of western Himalaya, extending from Kashmir to Kumaon. Its chief habitat is in the alpine and sub-alpine areas at elevations of 3000-3500 m, like Gulmarag, Kulanmar, Somamarg (Unyal et al., 2002). The tuberous roots of genus Aconitum contain alkaloids: benzoylmesaconine, meseaconitine, aconitine, hyaconitine, heteratins, heterophylline, heterophylline, heterophylline, heterophylline, atidine, isosilene, hetidine, hetisine and benzoylhetisine (Zhaochong et al., 2005; Pelliter et al., 1968) plant contain Alkaloids heteratins, heterophylline, heterophylline, heterophylline, atidine, isosilene hetidine, hetisine and benzoylhetisine (Zhaochong et al., 2005). The roots, which have been used mostly as poison than as drug, are now reported to possess significant antipyretic and analgesic properties and a high therapeutic index. The Aconitum alkaloids mesaconitine and 3 acetylacoonitine have been shown to possess anti inflammatory activity (Ameri et al., 1998). The plant is used for the treatment of diseases of the nervous system, digestive system, rheumatism and fever. Aconitum has biological and pharmacological activities such as anti-fungal, anti-bacterial, insecticidal and Brine shrimp cytotoxic activities (Arner et al., 2003). The plant possesses potent immunostimulant property (Atal et al., 1986). The root extract exhibit anti-viral activity against Spinach Mosaic Virus (SMV) (Putwardhan et al., 1990). It is also used for curing hysteria, throat infection, dyspepsia, abdominal pain and diabetes. In the indigenous system of medicine this plant is considered as valuable febrifuge, nerve tonic, especially in combating debility after malaria and in hemoplegia. In view of the continued popularity of Aconitum heterophyllum in indigenous as well as modern system of medicine coupled with unscientific and indiscriminate extraction from wild sources has reduced this high value plant species towards rarity and is already in red list (Dar et al., 2001). Organized cultivation of Aconitum is therefore necessary to ensure the quality and continuous supply of drug. Moreover, the tubers derived from seeds result in large genetic variability and are frequently infected with fungal diseases, mainly Verticilloium sp. (Pirone et al., 1978). Seedlings are plagued by their variation in quality and quantity, natural uniformity is not maintained throughout. In the past many programmes have been initiated for the

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in situ propagation of this endangered medicinal plant. However, they are limited by several factors. The potential for regeneration of medicinal plants out of their natural habitat is poor. The germination of seeds and establishment of seedlings is also poor (Khan and Khanum, 1998). The number of propagules produced by the natural methods is limited and insufficient for large-scale planting in the wild or under field conditions. Using in vitro and associated biotechnological interventions, the removal of such otherwise problems could be possible.

Tissue culture techniques have been used for endangered plants to generate large numbers of propagules, which can be reintroduced in their native habitat. It can enable the mass propagation of this herb from a minimum of plant material so that large quantities of biomass required for extraction of active constituents can be made available throughout the year, without causing further endangerment of the species. This study on Aconitum heterophyllum was taken up with a view to develop techniques for its in vitro multiplication.

MATERIALS AND METHODS

Fresh viable seeds of Aconitum heterophyllum were procured from the Gene bank of Regional Research Laboratory (CSIR), Srinagar, Kashmir in the year 2004-2005. Voucher specimen was deposited in the repository of RRL, Srinagar, (Voucher No. RRL/AC/Srinagar-2004). Seeds were washed with detergent and 2-4 drops of Tween-20 (Himedia) under running tap water, followed by final rinsing with double-distilled water. These seeds were soaked in double-distilled water for 2-3 days at 4°C in a refrigerator. Surface sterilization of soaked seeds was achieved by using 0.1% (w/v) mercuric chloride for 3-4 min, followed by three times rinsing with autoclaved double distilled water to remove all traces of sterilant. The sterilized seeds were then kept in the dark at 20±2°C on moist absorbent cotton in the petri plates. The germinated seedlings were inoculated on MS (Murashige and Skoog, 1962) basal media fortified with 3% sucrose. pH of the media was adjusted at 5.8 by using 0.1N NaOH and 0.1N HCl before getting the medium with 0.8% agar-agar type (Himedia). The media was finally dispensed into culture tubes, which were plugged and autoclaved for 20 min. at 1.5 lb pressure and 121°C temperature. These cultures were maintained at 25±2°C with 55-65% relative humidity and exposed to 16 hours photoperiod provided by cool fluorescent tubes (3000 lux).

RESULTS AND DISCUSSION

Seeds of Aconitum heterophyllum, cultured on moist absorbent cotton, resulted in full-sized seedling formation after 4 weeks of their culture (Fig. 1). Nodal explants were excised aseptically from these seedlings and cultured on different phytotrophic regimes, the effect of which is depicted in Table 1. Compact callus was formed on MS medium supplemented with various concentrations of 2,4-D (0.1-0.4 mg L⁻¹), with 40% response. With NAA (0.1 mg L⁻¹), single shoot, followed by root formation was obtained from the nodal bud. However, comparatively higher concentrations of NAA (0.6 mg L⁻¹) resulted in non-differentiating callus formation with 70% response.

Various BAP concentrations (0.10-0.50 mg L⁻¹) stimulated direct axillary shoot initiation, proliferation and elongation. The maximum number of shoots survived and grew best on BAP (0.25 mg L⁻¹) with 80% response (Fig. 2). However, higher concentration of BAP (0.5 and 1.0 mg L⁻¹) reduced the number of axillary shoots per explant. The combined interaction of BAP (0.1 mg L⁻¹) + 2, 4-D (0.1 mg L⁻¹) also resulted in shoot proliferation but with lower percentage of shoot formation. Profuse callus was formed on MS medium supplemented
Table 1: Morphogenic response of nodal explants of Acconium heterophyllum to various phytohormonal regimes

<table>
<thead>
<tr>
<th>Z4-D (mg L⁻¹)</th>
<th>BAP (mg L⁻¹)</th>
<th>NAA (mg L⁻¹)</th>
<th>Response</th>
<th>% response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.00</td>
<td>0.00</td>
<td>Green callus formed</td>
<td>40</td>
</tr>
<tr>
<td>0</td>
<td>0.00</td>
<td>0.10</td>
<td>Single shoot formed from nodal bud. Shoot adventitious roots were also formed at basal end of shoot</td>
<td>60</td>
</tr>
<tr>
<td>0</td>
<td>0.00</td>
<td>0.10</td>
<td>Friable Callus formation</td>
<td>70</td>
</tr>
<tr>
<td>0</td>
<td>0.10</td>
<td>0.00</td>
<td>Shoot proliferation</td>
<td>60</td>
</tr>
<tr>
<td>0</td>
<td>0.25</td>
<td>0.00</td>
<td>'de-'</td>
<td>80</td>
</tr>
<tr>
<td>0</td>
<td>0.50</td>
<td>0.00</td>
<td>'de-'</td>
<td>40</td>
</tr>
<tr>
<td>0</td>
<td>0.10</td>
<td>0.00</td>
<td>'de-'</td>
<td>60</td>
</tr>
<tr>
<td>0</td>
<td>0.05</td>
<td>0.00</td>
<td>White callus formed</td>
<td>60</td>
</tr>
<tr>
<td>0</td>
<td>1.00</td>
<td>0.00</td>
<td>Callus</td>
<td>70</td>
</tr>
<tr>
<td>0</td>
<td>0.50</td>
<td>0.25</td>
<td>Shoot formed from nodal bud</td>
<td>80</td>
</tr>
<tr>
<td>0</td>
<td>1.00</td>
<td>1.00</td>
<td>White nodular callus formation, shoot formation, multiple shoot formation, and multiple shoot regeneration via callus re-differentiation</td>
<td>90</td>
</tr>
</tbody>
</table>

*Data recorded after 4 weeks of culture

Table 2: Rooting response in Acconium heterophyllum after inoculation on MS media supplemented with different concentrations of auxins (observation recorded after 35-45 days)

<table>
<thead>
<tr>
<th>Media combinations supplemented with auxins (mg L⁻¹)</th>
<th>Mean No. of roots</th>
<th>Rooting in mean length of roots (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>MS</td>
<td>1.5</td>
<td>30-37</td>
</tr>
<tr>
<td>MS+IAA (0.2 mg L⁻¹)</td>
<td>1.3</td>
<td>30.37</td>
</tr>
<tr>
<td>MS+IAA (0.5 mg L⁻¹)</td>
<td>1.5</td>
<td>30-40</td>
</tr>
<tr>
<td>MS+IBA (1.0 mg L⁻¹)</td>
<td>1.0</td>
<td>40-43</td>
</tr>
<tr>
<td>MS+IBA+IAA (0.5+1.0 mg L⁻¹)</td>
<td>2.9</td>
<td>45-48</td>
</tr>
<tr>
<td>MS+IBA+IAA (1.0 mg L⁻¹)</td>
<td>1.8</td>
<td>26-27</td>
</tr>
</tbody>
</table>

*The data is based on 5 replicate cultures, while the experiment was repeated three times; mg L⁻¹ = milligram per liter

Fig. 4: In direct shoot proliferation

Fig. 5: Rooting

Fig. 3: I. Friable callus; II. Green callus

Fig. 6: Plant in field
by BAP (0.25 mg L\(^{-1}\)) + 2,4-D (0.5 mg L\(^{-1}\)) with 80% response (Fig. 3). The combined interaction of BAP (0.5 and 1.0 mg L\(^{-1}\)) + NAA (0.25 and 1.0 mg L\(^{-1}\)) resulted in compact callus formation at the basal ends, which was followed by multiple shoot regeneration and elongation on the same media with 90% response (Fig. 4). Direct root initiation and elongation was observed after sub-culturing of isolated shoots into MS basal media within 4 weeks of their culture period (Table 2). The rooting response of shoots was also initiated on MS basal media fortified with IAA (1.0 mg L\(^{-1}\)) after 4 weeks of culturing (Fig. 5). Complete plantlets (6-10 cm) were recovered after 6-8 weeks on rooting media. The healthy plantlets were deflasked and transferred into pots containing sand, soil and vermiculite mixture (1:1:1) (Fig. 6).

In the present study, the maximum multiple direct adventitious shoot regeneration and elongation was observed by culturing nodal segments of *A. heterophyllum* on MS media augmented with BAP (0.25 mg L\(^{-1}\)). The results are very much in conformity with other previous studies (Giri et al., 1993). Our results are also supported by the observations in liquid culture of *A. napellus* (Watad et al., 1995). Callus regeneration was observed at the basal cut ends of each explant, which is in agreement with the anther culture of *A. carmichaeli* (Hatano et al., 1987).

Combined effect of BAP (1.0 mg L\(^{-1}\)) + NAA (1.0 mg L\(^{-1}\)) on nodal segments promoted multiple indirect adventitious shoot regeneration and elongation. However, the combined interaction of BAP (1.0 mg L\(^{-1}\)) + 2,4-D (1.0 mg L\(^{-1}\)) on nodal segments showed low percentage of response. Such findings are strongly supported by those of Giri et al. (1993) who reported the callus formation on MS medium containing 2, 4-D, Kinetin, Coconut water and maintained on 1.0 mg L\(^{-1}\) level of NAA. Rooting of the elongated shoots was initiated on MS basal medium fortified by IAA (1.0 mg L\(^{-1}\)) 4 weeks after culturing. However, root formation results recorded by Giri and Watad in *A. heterophyllum* and *A. napellus* was obtained best on IBA (1.0 mg L\(^{-1}\)) and NAA (1.0 mg L\(^{-1}\)), respectively.

The detailed review of the earlier studies reveal that there is only scanty published data on organogenesis of this plant species. However, there are few published reports regarding liquid culture of *A. napellus*, *A. balfourii* and *A. carmichaeli*. (Watad et al., 1995; Hatano et al., 1987; Pandey et al., 2004 and Hatano et al., 1988).

Results of the study reveal that the protocol developed for them micro-propagation of *A. heterophyllum* has the potential to be reproduced and utilized for large-scale multiplication viz a viz conservation of this medicinal herb, an indigenous endangered medicinal plant. Friable callus formation was obtained on NAA (0.6 mg L\(^{-1}\)) (Fig. 3), but callus maintenance was difficult due to excessive leaching of apparent phenolic compounds. The problem was more acute on hormonal combinations of BAP (0.5 mg L\(^{-1}\)) and with 2, 4-D (0.25 mg L\(^{-1}\)).

Inclusion of anti-phenolic substances, such as ascorbic acid (10 mg L\(^{-1}\)) polyvinylpyrrolidone (pvp) 0.5% and activated charcoal (2%) could not overcome this problem. Hence callus induced on 2, 4-D supplemented medium was transferred to NAA (1.0 mg L\(^{-1}\)) containing medium. The cultures were sub-cultured after one week’s time, so as to prevent the leaching of exudates. The study indicates that *A. heterophyllum* populations in the North-western Himalaya are genetically diverse. At present, rate of its propagation is far less as compared to its exploitation. These species, or at least a significant proportion of its genetic diversity may be lost in near future, if appropriate measures are not taken for its conservation.

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REFERENCES


