IN VITRO SHOOT PROLIFERATION FROM APICAL AND NODAL EXPLANTS OF RAUWOLFIA SERPENTINA (L.) BENTH - AN ENDANGERED MEDICINAL PLANT

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ABSTRACT

Consequency was obtained for mass propagation of a highly endangered medicinal plant, Rauwolfia serpentina (L.) Benth through in vitro culture. Investigation had conducted on assessing the effect of different combinations of growth hormones at different levels of concentrations for high shoot proliferation on different explants. Apical and Nodal buds of young sprouts from selected plants were used as explants. Best shoot induction from apical buds was observed on MS basal medium supplemented with IBA 0.125 mg/l + BAP 1.0 mg/l in which 47% of shoot formation occurred. Similarly in case of nodal buds as explant best shoot induction was observed on MS basal medium supplemented with IBA 0.125 mg/l + BAP 1.0 mg/l in which 35% of shoot formation occurred. The technique developed can be effectively workout for extensive multiplication and enduring in vitro conservation of Rauwolfia serpentina. The special purpose of current vstudy was to identify most suitable media supporting competence necessary for large-scale propagation scheme to replenish the exhausting supply and to conserve the threatened species.

KEYWORDS: Rauwolfia serpentina, endangered plant, Shoot proliferation, in vitro.

INTRODUCTION

Rauwolfia serpentina is an important medicinal plant of the world. There is a great demand for its roots, which is the prime cause of its indiscriminate uprooting from wild sources where it grows abundantly. Mass scale collection of this plant from natural habitat by the
pharmaceutical industries as well as local Ayurvedic and Unani practitioners is leading to a depletion of this plant resource.\textsuperscript{[1]}

Due to its over exploitation from wild resources \textit{Rauwolfia serpentina} is currently listed in Appendix II of Convention on International Trade in Endangered Species.\textsuperscript{[2]} This has led to listing of this species as “endangered” by the International Union for Conservation of Nature and Natural Resources.\textsuperscript{[3]}

\textit{In vitro} cell and tissue culture is envisaged as a means for germplasm conservation to ensure the survival of endangered plant species, rapid mass propagation for large scale propagation and for genetic manipulation studies. Propagation by means of seeds to replenish the exhausting supply might prove ultimately even unwise because of its poor seed viability and very low germination percentages (25-50\%) that may be ascribed largely due to the presence of cinamic acid derivates in the seeds.\textsuperscript{[4]}

\textit{In vitro} propagation studies of different plant species have shown that the technique may be a solution for rapid propagation of such selected useful plant species and subsequent exploitation.\textsuperscript{[5]} It also has been found that ex-plants of an alkaloid producing plant, cultured \textit{in vitro} retain the capacity to synthesis alkaloids to that in the intact plant.\textsuperscript{[6]}

On the other hand \textit{in vitro} micro propagation has a number of advantages over sexual propagation. In sexual method, superior genotypes may be lost through recombination but \textit{in vitro} propagation can preserve superior gene combinations practically unaltered.\textsuperscript{[7]}

**MATERIALS AND METHODS**

**Collection and preparation of ex-plants**

The ex-plants were obtained from the different genotypes maintained in the germplasm bank of College of Forestry, Sirsi under the National Medicinal Plant Board Project. For direct regeneration, both nodal and apical segments of 1 - 2 cm size were taken from field grown plant and after following proper sterilization, were transferred to MS media supplemented with hormones under aseptic condition.

**Culture medium and culture condition**

The culture media tested were (Murashige and Skoog, 1962), with 3\% sucrose (w/v) and 0.8\% (w/v) agar. The pH of the medium was adjusted to 5.8 and solidified with 0.8\% (w/v) agar–agar prior to autoclaving at 121°C for 15 min. The cultures were incubated under...
controlled conditions such as 25 ± 2°C temperature, 60 ± 10% relative humidity, and 61/8-h photoperiod (PFD 50 μmol m−2 s−1) provided by white fluorescent lamps.

**Surface sterilization of ex-plants**

Explants were collected from field growing plants and thoroughly washed with running water for 30 min. Washed ex-plants were treated with 1.0% mild detergent (Tween-20) for 5 min and rinsed off thoroughly under tap water. This is followed by mercuric chloride treatment (0.1% w/v) for 2 min, and washing 2 - 3 times with distilled water. Thereafter, the ex-plants were treated with sodium hypochlorite (50%, v/v) for 2 min, and washing 2 - 3 times. Finally they were washed 4 - 5 times properly with sterile double distilled water and then placed on sterile filter paper sheets to remove moisture. Both mercuric chloride and sodium hypochlorite treatments were given inside the laminar flow chamber.

**RESULTS**

**Shoot proliferation from apical bud ex-plant**

Media containing 2,4-D, 0.125 mg/l + BAP, 1.0 mg/l produced only 1 shoot even after 10 days, 21 days and 30 days of observation. The number of nodes varied from 1 to 3 from 10 to 30 days. Media containing 2,4-D, 0.125 mg/l + BAP, 1.5 mg/l produced only 1 shoot which keeps on elongated till the 30 days of observation. Similarly the media containing 2,4-D, 0.250 mg/l + BAP, 1.5 mg/l produced only 1 shoot with 2 nodes till the 30 days of observation.

The media proliferated with hormonal combination of IBA, 0.125 mg/l + BAP, 1.0 mg/l produced large number of shoots which varied from 2 to 6 from 10 to 30 days of observation. Even though the media containing IBA, 0.125 mg/l + BAP, 1.5 mg/l produced 5 shoots till 30 days of observation but the growth rate of these produced shoots was quite slow. Moreover, the media containing IBA, 0.250 mg/l + BAP, 1.5 mg/l resulted in callus induction and the number of shoots produced varied from 2 to 4 till the 30 days of observation. The percent shoot formation was highest (47.14%) in media supplemented with IBA, 0.125 mg/l + BAP, 1.0 mg/l, followed by IBA, 0.125 mg/l + BAP, 1.5 mg/l (42.85%) and lowest (21.42%) was observed with the treatment of 2,4-D, 0.125 mg/l + BAP, 1.5 mg/l.

**Shoot proliferation from nodal bud ex-plant**

Media containing 2,4-D, 0.125 mg/l + BAP, 1.0 mg/l produced only 1 shoot. Similarly the media containing 2,4-D, 0.125 mg/l + BAP, 1.5 mg/l resulted into the formation of only 1
shoot. The media pertaining growth regulator combination of 2,4-D, 0.250 mg/l + BAP, 1.0 mg/l also engulfs only 1 shoot.

Along with this the MS media supplemented with IBA, 0.125 mg/l + BAP, 1.0 mg/l developed only 1 shoot with high shoot length 6.3 ± 0.2 cm along with high per cent shoot formation (35.71%). Media fortified with IBA, 0.125 mg/l + BAP, 1.5 mg/l results in the formation of only 1 shoot. Moreover the media containing IBA, 0.250 mg/l + BAP, 1.0 mg/l produced only 1 shoot which pertained low shoot length (1.8 ± 0.5).

**DISCUSSION**

**Effect of plant growth regulators on shoot proliferation from apical bud ex-plant**

In the present study, the auxin IBA and cytokinin BAP were supplemented to basal MS medium. Best growth of axillary shoots was obtained on MS containing IBA 0.125 mg/l + BAP 1.0 mg/l pertaining high shoot potentiality. Two to six vigorously growing shoots were developed on the ex-plant, which was due to the multiplication of the original bud. The number of shoots formed on this combination of phyto-hormones was greater than that of 2, 4-D and BAP. A cluster of multiple shoots was also developed on the apical bud ex-plants under the influence of IBA0.125 mg/l + BAP 1.5 mg/l. Similarly, Mathur et al. (1993) induced multiple shoots on apical bud segments from *in vitro* shoots of *Rauwolfia serpentina*, cultured on MS medium containing 1.0 mg/l BAP and 0.1 mg/l NAA.

Low concentration of IBA (0.125 mg/l) supplied along with high or low concentration of BAP (1.0 mg/l or 1.5 mg/l) had no stimulatory or inhibitory effect on shoot regeneration, but using both IBA as well as BAP at high concentration *i.e.* 0.250 mg/l and 1.5 mg/l respectively leads to reduction in shoot regeneration frequency and callogenesis was promoted. Similarly, Rajkarnikar et al. (2000) also found that BAP 1 mg/l and 2 mg/l with 0.1 mg/l of NAA showed the best result and also reported that in higher concentration of BAP callusing occurred at the top of ex-plants.

The enhanced rate of multiple shoot induction in cultures supplemented with BAP and IBA may be attributed to increased rate of cell division and production of several primordial outgrowths induced by cytokinin (BAP) in the axillary meristematic zone of the ex-plant tissues. However, at higher concentration of 2, 4-D (0.250 mg/l) along with 1.5 mg/l BAP, bud multiplication frequency got reduced and only a single shoot was developed. Similar results have been reported by Ilahi and Rahim, (2007) for shoot induction of *Rauwolfia serpentina*. 
Effect of plant growth regulators on shoot proliferation from nodal bud ex-plant

In the present study 2, 4-D 0.125 mg/l + BAP 1.0 mg/l was found sufficient to produce more shoots and the interactions of 2, 4-D at higher concentrations i.e. 0.250 mg/l did not favour numerous shoot proliferation, which was significantly lower than that with combination of 2, 4-D and BAP at a concentration of 0.125 mg/l and 1.5 mg/l respectively. In a similar experiment, Kataria and Shekhawat (2005) had found 20-25 fold increment in multiple shoot production using BAP 10 μM and IAA 5 μM.

MS media supplemented with IBA 0.125 mg/l + BAP 1.0 mg/l was the best combination for inducing shoots of *R. serpentina* by nodal bud ex-plant. On the other hand, the same combination IBA + BAP but at a concentration of 0.250 mg/L and 1.0 mg/L showed poor response with low percent shoot formation and also with low shoot length. It appears that explants possess poor ability to utilize higher concentration of IBA from the medium. In earlier reports too, the shoot length in *Rauwolfia serpentina* was maximized with the combined action of BAP and NAA, whereas BAP alone or its interactions with other hormones significantly decreased shoot length (Baksha *et al.*, 2007).
Table: 1. Effect of growth regulators on shoot proliferation of *Rauwolfia serpentina* apical bud ex-plant

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Growth Regulator</th>
<th>Observation after inoculation</th>
<th>Percent (%) Shoot Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MS media + 2,4-D 0.125 mg/l + BAP 1.0 mg/l</td>
<td>1 shoot and 1 node were found per ex-plant</td>
<td>35.71</td>
</tr>
<tr>
<td>2</td>
<td>MS media + 2,4-D 0.125 mg/l + BAP 1.5 mg/l</td>
<td>Elongation of shoot with 1 node were found per ex-plant</td>
<td>21.42</td>
</tr>
<tr>
<td>3</td>
<td>MS media + 2,4-D 0.250 mg/l + BAP 1.5 mg/l</td>
<td>1 shoot and 1 node were found per ex-plant</td>
<td>30.80</td>
</tr>
<tr>
<td>4</td>
<td>MS media + IBA 0.125 mg/l + BAP 1.0 mg/l</td>
<td>2 shoots were proliferated per ex-plant</td>
<td>47.14</td>
</tr>
<tr>
<td>5</td>
<td>MS media + IBA0.125 mg/l + BAP 1.5 mg/l</td>
<td>2 shoots were found per ex-plant</td>
<td>42.85</td>
</tr>
<tr>
<td>6</td>
<td>MS media + IBA0.250 mg/l + BAP 1.5 mg/l</td>
<td>Callus induced and 2 shoots were proliferated on callus of each ex-plant</td>
<td>28.57</td>
</tr>
</tbody>
</table>

Table: 2. Effect of growth regulators on shoot proliferation of *Rauwolfia serpentina* nodal bud ex-plant

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Growth Regulator</th>
<th>Observation after 10 days</th>
<th>Observation after 21 days</th>
<th>Observation after 30 days</th>
<th>Per cent Shoot Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MS media + 2,4-D 0.125 mg/l + BAP 1.0 mg/l</td>
<td>1.0 ± 0.2</td>
<td>2.3 ± 0.5</td>
<td>1.25 ± 0.5</td>
<td>2.25 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>MS media + 2,4-D 0.125 mg/l + BAP 1.5 mg/l</td>
<td>2.0 ± 0.2</td>
<td>2.1 ± 0.6</td>
<td>3.0 ± 0.2</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>MS media + 2,4-D 0.250 mg/l + BAP 1.0 mg/l</td>
<td>2.0 ± 0.4</td>
<td>3.6 ± 0.8</td>
<td>3.5 ± 1.1</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>4</td>
<td>MS media + IBA 0.125 mg/l + BAP 1.0 mg/l</td>
<td>2.0 ± 0.0</td>
<td>3.2 ± 0.2</td>
<td>3.0 ± 0.5</td>
<td>4.75 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>MS media + IBA 0.125 mg/l + BAP 1.5 mg/l</td>
<td>2.25 ± 0.5</td>
<td>1.8 ± 0.4</td>
<td>3.25 ± 0.3</td>
<td>4.25 ± 0.0</td>
</tr>
<tr>
<td>6</td>
<td>MS media + IBA0.250 mg/l + BAP 1.0 mg/l</td>
<td>1.25 ± 0.0</td>
<td>1.00 ± 0.2</td>
<td>2.5 ± 0.8</td>
<td>3.25 ± 0.2</td>
</tr>
</tbody>
</table>
Fig. 1 Apical bud shoots in MS media + IBA 0.125mg/l + BAP 1.0 mg/l

Fig. 2 Apical bud shoots in MS media + IBA 0.125mg/l + BAP 1.5 mg/l

Fig. 3 Apical bud shoots in MS media + IBA 0.250 mg/l + BAP 1.5 mg/l
Fig. 4 Nodal buds shoot in MS media + 2,4-D 0.125mg/l + BAP 1.5 mg/l

Fig. 5 Nodal buds shoot in MS media + 2,4-D 0.250mg/l + BAP 1.0 mg/l

Fig. 6 Nodal buds shoot in MS media + IBA 0.125mg/l + BAP 1.0 mg/l
CONCLUSION
MS basal medium supplemented with IBA 0.125 mg/l + BAP 1.0 mg/l revealed high shoot induction from apical as well as nodal bud explants.

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