In vitro propagation of an endemic umbellifer, Hydrocotyle conferta

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Received 19 April 2006; revised 6 February 2007; accepted 5 April 2007

A protocol for in vitro propagation of Hydrocotyle conferta Wight (Apiaceae) through axillary bud multiplication was established. Murashige and Skoog (MS) medium with 6.66 μM N6-benzyladenine (BA) and 5.37 μM α-naphthalenacetic acid (NAA) was best suited for axillary bud multiplication including a mean of 21 shoots/node. Excision and culture of the nodal segments from the in vitro shoots on fresh medium with same concentrations of BA and NAA facilitated development of more than 25 shoots/node. Subsequent cultures enhanced the rate of shoot proliferation. The developed shoots rooted best on half strength MS medium with 0.54 μM NAA. Plantlets established in pots exhibited 95% survival.

Keywords: axillary bud multiplication, Hydrocotyle conferta, endemic umbellifer

IPC Code: Int. Cl. 8 A01H4/00

Introduction

Hydrocotyle conferta Wight (Apiaceae) is chiefly valued for its edible nature and is used as a flavouring agent. It is a threatened herb generally endemic to western Ghats of South India1. In recent years, the rapid pace of deforestation in the western Ghats has depleted the wild population of H. conferta and hampered its natural regeneration potential. The natural regeneration as well as conventional propagation of this plant has also been attributed to several other factors2. Consequently, it has been enlisted as a rare species3. Hydrocotyle rotundifolia is used as a substitute of Centella asiatica in North Indian regions for extraction of asiaticoside and madecassoside, which are being currently used in the treatment of leprosy, eczema, skin lesions, psoriasis, intestinal ulcers, tuberculosis and venous diseases4. H. conferta is also a possible alternative source of asiaticoside and madecassoside, but it is distributed in a too small geographic region of western Ghats with few countable populations. Lack of ethnobotanical knowledge and unavailability of H. conferta has led to lacunae in the knowledge of uncharacterized compounds. Therefore, there is a need to conserve and propagate this herb for its survival. The application of plant tissue culture in ex situ conservation of rare and endangered taxa, including medicinal plants, has long been recognized5. In vitro propagation of rare species is generally undertaken to increase the number of individuals available. The present paper describes in vitro multiplication of H. conferta through axillary bud multiplication.

Materials and Methods

Nodal segments were collected from the tender parts of mature plants grown in pots of the Botanical Garden of Sri Krishnadevaraya University. They were washed under running tap water followed by a wash with surfactant Tween 20 (5% v/v) for 5 min. After repeated washes in double distilled water, surface sterilization was done with mercuric chloride (0.5% w/v) solution for 7-10 min. The sterilized segments were then washed thoroughly with sterile double distilled water and cut into appropriate size, and cultured on sterile nutrient medium. Murashige and Skoog6 medium used as the basal medium was supplemented with 2% (w/v) sucrose and gelled with 0.8% (w/v) agar (Merck). Different growth regulators (BA, Kn, NAA, IAA, IBA) at different concentrations either alone or in combination were added to the medium. Half strength MS medium supplemented with auxin (NAA/IAA/IBA) alone was tried for in vitro rooting. The pH of the medium was adjusted to 5.8 before autoclaving at a pressure of 1.06 kg cm2. All the cultures were incubated at 25±2°C with 16/8 h photoperiod under white fluorescent tubes (25 μmol m-2 s-1). Fifteen cultures were raised for each treatment and all experiments were repeated twice. Means were compared using Duncans multiple range

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test. Rooted shoots were directly transferred to small pots containing peat moss and garden soil (1:1) and subsequently to the field.

**Results and Discussion**

Nodal explants cultured on MS basal medium induced a mean of two shoots (Table 1). All concentrations of BA/Kn either alone or in combination facilitated axillary bud initiation. BA was the most effective cytokinin for the axillary bud initiation and subsequent proliferation. BA alone at 6.66 μM induced a mean of 12 shoots per nodal explant (Table 1; Fig. 1a). A similar result was reported in some Apiaceae members like *Bupleurum kaoi* and *Heracleum candicans*. Kn supplemented medium resulted in less number of shoots, but gave shoots with longer internodes (Fig. 1c). BA with Kn induced highest shoot proliferation in *H. rotundifolia*.

BA at 6.66 μM in combination with 5.37 μM NAA was most effective for axillary bud multiplication.

**Table 1 — Axillary bud multiplication of H. conferta on MS medium supplemented with different growth regulators**

<table>
<thead>
<tr>
<th>Growth regulators (μM)</th>
<th>BA</th>
<th>Kn</th>
<th>IBA</th>
<th>IAA</th>
<th>NAA</th>
<th>Per cent</th>
<th>*Mean no. response of shoots/node</th>
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<tr>
<td>Growth regulator free</td>
<td>50</td>
<td>2.84</td>
<td>70</td>
<td>6.1⁸</td>
<td>90</td>
<td>8.8⁴</td>
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<td>2.22</td>
<td>90</td>
<td>12.6⁴</td>
<td>6.66</td>
<td>90</td>
<td>2.22</td>
<td>70</td>
<td>6.1⁴</td>
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<tr>
<td>4.44</td>
<td>100</td>
<td>10.1⁴</td>
<td>8.87</td>
<td>90</td>
<td>7.2⁴</td>
<td>70</td>
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<td>6.66</td>
<td>2.85</td>
<td>90</td>
<td>6.9⁴</td>
<td>6.97</td>
<td>80</td>
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<td>9.29</td>
<td>2.85</td>
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<td>6.9⁴</td>
<td>9.29</td>
<td>80</td>
<td>3.4⁴</td>
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<td>23.20</td>
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<td>90</td>
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<td>3.4⁴</td>
<td>90</td>
</tr>
<tr>
<td>6.66</td>
<td>100</td>
<td>19.7⁴</td>
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<td>5.71</td>
<td>90</td>
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<td>5.71</td>
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*Data represents the mean of 15 replicates. Mean followed by different letters are significantly different at 5% level. Culture period 40 d.*

which developed a mean of 21 shoots per nodal explant (Table 1; Fig. 1b). A synergistic effect of BA in combination with an auxin has been demonstrated in a few Apiaceae members, viz. *Anethum graveolens* and *C. asiatica*. Excision of the nodal segments from these *in vitro* shoots and its culture facilitated the development of more than 25 shoots/node. During subculture, basal axillary buds of the developed shoots also underwent initiation. Enhanced shoot multiplication in subsequent cultures has been reported in *Gymnema sylvestre*, *Holostemma ada-kodien*, *Ceroplastia candelabrum*, *Solanum trifoliatum*, and *Spilanthes acmella*. However, in *A. graveolens* repeated subcultureing did not increase shoot proliferation.

Half strength MS growth regulator free medium induced more roots compared to full strength MS. Half strength MS growth regulator free medium is superior to full strength MS medium for *in vitro* root induction in some Apiaceae members like *C. was most effective for axillary bud multiplication, Eryngium foetidug.* Of the three auxins, NAA was found best for inducing roots, followed by IAA and IBA. IBA was poor in rooting

![Fig. 1 — *In vitro* propagation of H. conferta: a, Axillary bud initiation from nodal explant on MS medium containing 6.66 μM BA; b, Axillary bud multiplication on MS medium containing 6.66 μM BA+5.37 μM NAA; c, Axillary bud multiplication on MS medium containing 6.97 μM Kn; d, *In vitro* rooting on basal ½ MS medium containing 2% sucrose + 0.54 μM NAA; e, Hardened *in vitro* raised plant (after 2 weeks).](image-url)
and was characterized by callus formation. Half strength MS medium with 0.54 μM NAA developed a mean of 17 roots/shoot (Table 2; Fig.1d). Effectiveness of NAA in rooting has been reported in Physalis peruviana, and field conditions grew well and exhibited morphological characters similar to wild plants. They developed flowers and fruits normally. Thus, the present protocol is efficient to propagate this endemic species and to keep it off from extinction.

Acknowledgement
The first author is thankful to the Department of Biotechnology, New Delhi for financial assistance through DBT-Post Doctoral Fellowship.

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