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RESEARCH ARTICLE

Micropropagation and multiple shoot formation from nodal explants of *Hemidesmus indicus* (L.) *R.Br.* - A rare medicinal plant

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Abstract

Hemidesmus indicus R.Br. (Common name Anantamula, Ksheerini, Sarsaparilla) belongs to the family Asclepiadaceae. It is one of the rare medicinal & aromatic plant of South Asia. The plant is used to cure leprosy, leucoderma, itching and skin disease, asthma, bronchitis, leucorrhoea, dysentery, piles, syphilis and paralysis. It promotes health and is used in treatment of diseases caused by vitiated blood**.** The present study deals with the development of an efficient protocol for regeneration of *Hemidesmus indicus R.Br*. by using node segment as explants. The nodal explants were cultured on Murashige and Skoog's (MS) medium supplemented with different concentrations of growth hormones. The explants were cultured for 4 weeks on MS medium supplemented with different concentrations and combinations of BAP (6-Benzylaminopurine) and KN (6-Furfurylaminopurine) for shoot initiation and multiplication. Highest shoot induction (90%) and multiple shoot induction (09%) were observed on MS medium supplemented with 3.0 mg/L BAP with 02-04 shoots/explants. Average shoot length observed was 2cm. 77.7% rooting was observed in the MS medium containing 2.0 mg/L IAA (Indole-3-acetic acid) with root length 01-04 cm. The procedure reported in this study suggests that multiplication *via* tissue culture can be a commercially feasible method for *Hemidesmus* propagation. It would be helpful in providing uniform and consistent plant material for investigation of important secondary metabolites produced by this species, as well as its use as medicinal & aromatic plants.

Introduction

Hemidesmus indicus R.Br. (Anantamul) belongs to the family Asclepiadaceae is one of the most widely used medicinal plants in India, well known for its medicinal values. The plant is used to cure leprosy, leucoderma, itching, and skin disease, asthma, bronchitis, leucorrhoea, dysentery, piles, syphilis and paralysis, promotes health and cures all kinds of diseases caused by vitiated blood (Kirtikar and Basu, 1987). The plant is rare and getting endangered (Rahman, 2001). It is climbing slender plant. The root is long, rigid, cylindrical, little branched, a brownish corky bark, furrowed with annular cracks. Externally it has been applied as a poulitice to boils, swellings and other painful parts. The roots are harvested in autumn and dried for later use. Huge quantities of plant materials were imported for the manufacture of Ayurvedic, Unani and Homeopathic medicines (Chatterjee and Sastry, 2000).

Hemidesmus indicus is of economic interest for its wide-ranging pharmacological activity and one of the major constraints in utilizing natural population is the existence of plant-to-plant chemo variability. The fragrance of roots contains 2-hydroxy-4-methoxy benzaldehyde (91%) and ledol (4.5%), which are isolated in pure form, as the major constituents. Secondary metabolites of pharmaceutical values can be harvested from medicinal plants by tissue culture and hemidesmus acid, smilasperic acid from the cell suspension culture (Nagarajan *et. al*., 2001,

Ramachandra Rao, 2002, Broun, 2004). A standard protocol to induce multiple shoots in culture may provide a more homogenous source of plants. The present study deals with the development of an efficient protocol for micropropagation of *Hemidesmus indicus R.Br*. by using node segment as explants.

Materials and Methods

Plant materials

Hemidesmus indicus plants were collected from forest nursery Rishikesh, Uttrakhand. Young, healthy and disease free portion of the branches were selected and used as explants. Healthy nodal explants were selected and washed thoroughly under running tap water to wash off the dust present on the surface. The explants were then washed with few drops of Tween-20 for 05-10 min in tap water. These explants were sterilized with 0.5% of $HgCl_2$ solution for 05 min. followed by washing with sterile distilled water. After sterilization, the explants were transferred to the culture medium.

Culture media & Culture Conditions

MS medium supplement with 3% sucrose and gelled with 0.8% (w/v) agar (Himedia laboratories, India) was used. The pH of the medium was adjusted to 5.7-5.8 by 1N NaOH or 1N HCl. The media were steam sterilized in an autoclave under 15 psi and 121°C for 20 min. Inoculated cultures were incubated in the culture room at controlled environment of the temperature of 25±4°C of 16 hours photoperiod of 2000 lux and dark conditions.

Table-1 Media composition

Media	Composition
$MSB_{0.5}$	MS + BAP 0.5 mg/l
MSB_1	MS + BAP 1.0 mg/l
MSB_2	MS + BAP 2.0 mg/l
MSB_3	MS + BAP 3.0 mg/l
$MSB_1+K_{0.5}$	MS + BAP 1.0 mg/l + KN 0.5 mg/l
MSB_1+K_1	MS + BAP 1.0 mg/l + KN 1.0 mg/l
MSB_1+K_2	MS + BAP 1.0 mg/l + KN 2.0 mg/l
MSB_1+K_3	MS + BAP 1.0 mg/l + KN 3.0 mg/l
$MSI_{0.5}$	MS + IAA 0.5 mg/l
$MSI_{1.0}$	MS + IAA 1.0 mg/l
$MSI_{1.5}$	MS + IAA 1.5 mg/l
$MSI_{2.0}$	MS + IAA 2.0 mg/l
$MSI_{0.5}$	MS + IBA 0.5 mg/l
$MSIB_{1.0}$	MS + IBA 1.0 mg/l
$MSIB_{1.5}$	MS + IBA 1.5 mg/l
$MSIB_{2.0}$	MS + IBA 2.0 mg/l

Shoot initiation and multiplication

MS basal medium supplemented with Benzyl amino purine (BAP) individually and combination with Kinetin were used for shoot initiation. All the cultures were transferred to fresh medium after 2-3 weeks for further rapid shoot multiplication.

Root formation

For root induction, excised micro shoots (1-2 cm length) were transferred to MS basal medium supplemented with different concentration of IAA and IBA. One excised shoot was placed in each culture vessel having 20-25 ml of the culture media. The cultures were maintained by regular subcultures at 4- week intervals on fresh medium with the same composition.

Results and Discussion

For shoot induction nodal explants were cultured on MS basal medium (Murashige & Skoog medium) with growth hormone BAP and KN for induction of shoots. The cytokinin BAP was found to be superior and more effective than KN, for inducing shoot development as well as shoot multiplication from nodal explants. Among the various concentrations used in this study; BAP 3.0 mg/L proved to be optimum for initiation of shoots in which about 2-4 shoots were produced after 4 weeks. The percentage of shoot induction response is

observed 90% and shoots length 2 cm (Figure 1, Figure 1^{A} Table 2). Multiple shoot response was 83% in the presence of BAP 1 mg/L + KN 2.0 mg/L while maximum number of 02- 06 shoots after 4 weeks of culture having shoot length 2-3 cm (Figure 1^{B}, Table 2). 75-77.7% rooting frequency was observed in the medium containing 2.0 mg/L IAA+ IBA 1.0 mg/L) root length 01-03 cm. (Figure 1^{C}, Table 3).

The procedure reported in this study suggests that tissue culture will be a commercially feasible method for *Hemidesmus indicus* propagation**.** Micropropagation is more rapid, continuous and efficient than propagation via conventional cutting because it can supply uniform and consistent plant material for investigation of important secondary metabolites produced by this species, as well as its use as medicinal plants.

Table-2. Multiple shoot production from nodal explants of *Hemidesmus indicus*

S. No.	Plant growth hormones (mgl^{-1})		No. of explants inoculated	No. of explants shoot responded	Shooting (%)	No. of shoots per explants	Average Shoot length (cm)
	BAP	KN					
1	0.5	-	22	12	54.5	1	1.0
2	1.0	-	08	07	87.7	1-2	1.5-2.0
3	2.0	-	12	09	75.0	1-2	1.0-2.0
4	3.0	-	10	09	**90.0**	2-4	1.0
5	1.0	0.5	15	12	80.0	1-2	1.5
6	1.0	1.0	15	11	73.3	2-3	1.0
7	1.0	2.0	18	16	88.8	2-6	2.0
8	1.0	3.0	15	13	86.6	1-2	1.0-2.0

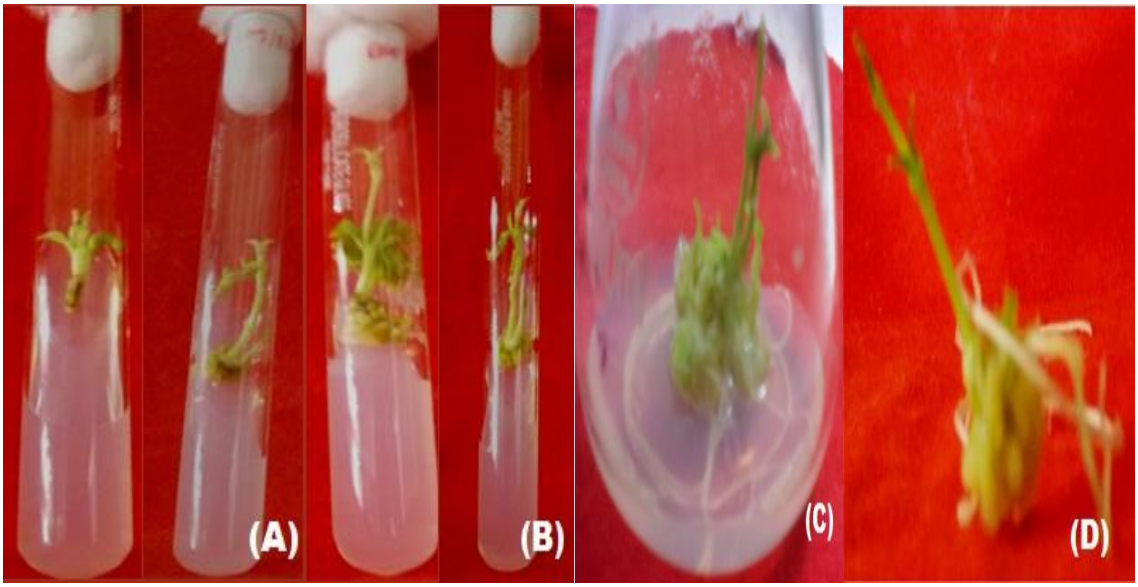

Fig. - 1 A-D: In vitro regeneration of *Hemidesmus indicus* (L.) *R.Br.* from nodal explants

A- Shoot initiation from nodal explants on MS + $3mgl^{-1}$ BAP.

B- Multiple shoot regeneration from nodal explants on MS + $3mgl^{-1}$ BAP.

C- Rooting from regenerated shoots on MS + $2mgl^{-1}$ IBA.

D- Complete regenerated plants.

Table-3. Rooting from regenerated shoot of *Hemidesmus indicus*

S. No.	Plant growth hormones (mgl^{-1})		No. of plants for rooting	No. of plants rooted responded	Rooting (%)	No. of roots per shoot	Average Root length (cm)
	IAA	IBA					
1	0.5	-	12	09	75.0	2-3	0.5-2.0
2	1.0	-	07	03	42.8	2-3	0.5-3.0
3	1.5	-	09	04	44.4	1-3	2.0-4.0
4	2.0	-	09	07	**77.7**	2-5	1.0-3.5
5	-	0.5	12	05	41.6	2-4	1.0-2.0
6	-	1.0	11	08	72.7	1-2	0.5-4.0
7	-	1.5	16	05	31.2	2-3	3.0-5.0
8	-	2.0	13	07	53.8	2-4	1.0-3.5

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