J. Plant Biochemistry & Biotechnology Vol. 12, 167-169, July 2003

Short Communication

In Vitro Method for Propagation of *Centella asiatica* (L) Urban by Shoot Tip Culture

Sangeeta Nath* and Alak K Buragohain

Department of Molecular Biology and Biotechnology, Tezpur University, Napaam, Assam 784 028, India

A rapid clonal propagation system has been developed for the medicinally important herb *Centella asiatica* (L) Urban by shoot tip (2-3 cm long) culture. The shoot tips isolated from mature plants were inoculated on MS medium incorporated with BA alone or in combination with NAA and Kn. The optimum number of shoots (3.38) with optimum number of leaves per shoot (4.25) were attained on MS medium supplemented with 4.0 mg l^{-1} BA and 0.1 mg l^{-1} NAA. On transferring the microshoots on full strength MS medium supplemented with various concentrations of IBA (1.0-3.0 mg l^{-1}) and NAA (0.5-2.0 mg l^{-1}), profuse rooting (46.8 per shoot) was obtained in MS basal medium with 2.0 mg l^{-1} IBA with root length of 19.7 cm. Well rooted plantlets were acclimatized successfully by adjusting the temperature and humidity for 3-4 weeks after transfer to pots filled with sterilized vermiculite soil : sand (1:1)mixture. This micropropgation protocol could be useful for raising a stock of genetically homogenous material for field cultivation within a very short period.

Key words: *Centella asiatica,* shoot tip, clonal propagation, Mandookaparni.

Centella asiatica (L) Urban is a member of Apiaceae family, commonly known in India as 'Indian pennywort' or 'Mandookaparni'. In traditional system of Indian medicine, *Centella asiatica* is a nervine tonic and is used in the treatment of leprosy, asthma, bronchitis, dropsy, leucorrhea, skin disease and urethritis (1). The wound healing activity of the species is ascribed to a triterpenoid saponin, asiaticoside while, antitumour properties are attributed to madecassic acid. *Centella* extracts have antitumour activity *in vitro* and *in vivo* (2).

Considering its medicinal properties and overexploitation from natural population the requirement for application of tissue culture techniques in the rapid multiplication of elite clones and germplasm conservation is a crucial prerequisite. Moreover, a stable supply of the bioactive secondary products has become a utmost priority. The present study was therefore, attempted to reproduce a rapid method for multiplication of the species by shoot tip culture to meet the requirement of the pharmaceutical industries. Earlier regeneration has been derived from leaf derived callus (3,4), stem segments (3) and nodal segments of *Centella asiatica* (5).

Plants of *Centella asiatica* were collected from in and around the Tezpur University Campus, Napaam. The plants were washed throroughly under running tap water for 30 min. The shoot tips (2-3 cm long) were then excised with a sharp razor and soaked in a beaker with detergent solution (1% Teepol) for about 30 min and washed thoroughly under running tap water. Surface sterilization of the shoot tips was done asceptically with 0.01% $HgCl_2$ (Himedia India) for 5 min followed by a final 3-4 rinses with sterile double distilled water. The explants were cultured on MS (6) medium supplemented with BA alone or in combination with NAA and Kn (Table 1) for shoot regeneration and various IBA/NAA combinations in full strength MS medium for root differentiation (Table 2). The cultures were incubated at 25 ± 2°C under 16 h photoperiod of 3000 lux intensity and 55-60% RH for shooting and under completely dark condition for rooting. Rooted plantlets were transferred to paper cups containing autoclaved vermiculite soil:sand (1:1). The plantlets were irrigated with MS basal liquid media devoid of Myo-inositol and sucrose at every 3 day interval. To maintain the humidity the pots were kept covered with polythene bags and maintained under 16 h photoperiod of 3000 lux intensity at 25 ± 2°C for 4 weeks before it was transferred to the field.

*Corresponding author. E-mail: sangeetanath15@rediffmail.com

Abbreviations: MS, Murashige and Skoog; NAA, α-Naphthalene acetic acid; Kn, Kinetin; BA, Benzyladenine; BAP, 6-benzylamino-purine; IBA, Indole-3-butyric acid; 2,4-D, 2,4- dihydrophenoxy-acetic acid; RH, relative humidity; RBD, Randomised Block Design; ANOVA, Analysis of Variance; DMRT, Duncan Multiple Range Test.

Data were analysed following Randomised Block Design (RBD). Each mean was based on eight replicates repeated five times each. The analysis of variance (ANOVA) appropriated for the design was carried out to detect the significance of differences among the treatment means. The treatment means were compared using Duncan Multiple Range Test (DMRT) at 5 % probability level according to Gomez and Gomez (7)

Shoot regeneration from *Centella asiatica* using shoot tip explants were carried out in MS medium supplemented with various concentration of BA alone or in combination with NAA and Kn (Table 1). Analysis of variance revealed significant effect of treatments(p<0.05) for number of shoots per responding explant, mean shoot length and average number of leaves per shoot. Shoot tips cultured in MS medium with BA alone showed increased shoot length though the frequency of shoots per responding explant and average number of leaves is more in cultures with BA (4.0 mg l^{-1}) and NAA (0.1 mg l^{-1}). An increase in the level of BA and NAA was found to promote callus formation as was previously found by Banerjee *et al* (4) when leaf was used as explant for shoot regeneration. Even Kn (4.0 mg l^{-1}) in combination with NAA (0.10 mg l^{-1}) have marked influence on shoot length and average number of leaves per shoot, though the number of shoots was less in comparison to BA and NAA .The superiority of BA over Kn in multiple shoot induction was also reported in a number of medicinal plants (8,9). A comparison of means by DMRT revealed that the optimum number of shoots (3.38) with optimum number of leaves per shoot (4.25) was attained on MS medium containing 4.0 mg l^{-1} BA and 0.1mg l^{-1} NAA (Fig. 1 A,B). Shoots with 3-4 nodes were subcultured every four weeks with a combination of 4.0 mg l^{-1} BA and 0.1mg l^{-1} NAA (Fig. 1 C). Elongated shoots of 6-7 cm in length were separated and cultured individually on full strength MS medium supplemented with various concentrations of IBA (1.0-3.0 mg l^{-1}) and NAA(0.5-2.0 mg l^{-1}) alone (Table 2).

Table 1. Analysis of variance and comparison by DMRT of the effects of BA, NAA and Kn concentrations on the number of shoots, mean shoot length and average number of leaves per shoot in *Centella asiatica*

Plant growth regulators (mg l^{-1})			Mean		
BA	NAA	Kn	Number of shoots per responding explants	Shoot length (mm)	Number of leaves per shoot
1.0	0.0	0.0	3.25^{a}	0.98^{a}	3.25^{ab}
2.0	0.05	0.0	1.50^{b}	0.72^{ab}	2.00^{cd}
3.0	0.05	0.0	2.50^{a}	0.51^{b}	2.38^{bc}
4.0	0.10	0.0	3.38^{a}	0.67^{ab}	4.25^{a}
0.0	0.0	1.0	1.00^{b}	0.57^{ab}	1.38^{cde}
0.0	0.10	2.0	0.75^{b}	0.44^{bc}	1.13^{de}
0.0	0.10	3.0	0.63^{b}	0.08^{c}	1.13^{de}
0.0	0.10	4.0	1.38^{b}	0.71^{ab}	3.63^{a}

Each mean is based on eight replicates each of which is repeated five times. Data recorded after four weeks of culture initiations. Each treatment mean followed by the same letter were not significantly different from each other (p<0.05) according to the Duncan Multiple Range Test (DMRT).

In the present investigation micro-shoots were directly transferred to rooting media. No separate media was needed for shoot elongation by which plantlet formation could be achieved in two steps only. Even shoots induced from nodal explants on the medium containing optimum BA and NAA medium failed to elongate rapidly and required a transfer to MS media supplemented with low level of BA (5). Analysis of variance showed significant effect on number of roots per shoot and mean root length. Profuse rooting was obtained in full strength MS medium supplemented with 2 mg l^{-1} IBA (Fig. 1 D). MS basal medium also showed minimum number of roots per shoot (4.8) with a mean root length of 10.5 cm. A comparison by DMRT revealed optimum number of roots per shoot (46.8) and mean root length (19.7cm) in MS medium containing

Table 2. Analysis of variance and comparison by DMRT of the effects of IBA and NAA concentrations on the number of roots per shoot and mean root length in *Centella asiatica*

Plant growth regulator (mg l^{-1})		Mean	
IBA	NAA	No. of roots per shoot	Root length (cm)
1.0	0	9.4^{b}	12.5^{b}
2.0	0	46.8^{a}	19.7^{a}
3.0	0	4.0^{b}	12.5^{b}
0	1.0	7.4^{b}	6.5^{c}
0	2.0	0.2^{b}	1.0^{d}
0	0.5	0.2^{b}	4.5^{c}
0	0	4.8^{b}	10.5^{b}

Each mean is based on five replicates each of which is repeated five times. Data recorded after four weeks of transfer into the rooting media. Treatment means followed by the same letter were not significantly different from each other(p<0.05) according to the Duncan Multiple Range Test (DMRT).