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MICROPROPAGATION OF *Centella asiatica* L. AN IMPORTANT MEDICINAL HERB

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ABSTRACT

High frequencies of multiple shoot regeneration were achieved from shoot tip explants cultured on MS media fortified with 4.0 mgl^{-1} BAP + 0.1 mgl^{-1} NAA. On an average 10.2±0.38 shoots per explant were obtained. The regenerated shoots were rooted in MS medium supplemented with 1.0 mgl^{-1} IBA. The *in vitro* grown plantlets were acclimatized and successfully transferred to natural condition with 80% survival. A reproducible protocol was established for *in vitro* propagation through multiple shoot induction of *Centella asiatica* L. an important medicinal herb having high medicinal value.

Key words : Micropropagation, Shoot tip, *Centella asiatica*

INTRODUCTION

Centella asiatica L. is a valuable medicinal herb belonging to the family Apiaceae. It is distributed throughout the tropical and subtropical countries like Bangladesh, India and Srilanka. In Bangladesh this plant is known as Thankuni (Huq, 1986). It is an evergreen perennial creeping herb with hollow or solid stem, alternate leaves, and epigynous, small, bisexual or staminate flowers, commonly found in moist place (Oyedeji and Afolayan, 2005). The plants possess antileprotic, antifilarial, antifeedant, adaptogenic, antiviral, antibacterial properties (Gurib-Fakin *et al*., 1997) and also anti-tumour activity (Babu *et al*., 1995). It is also reported to possess insecticidal (Stuart, 1982) and mutagenic properties (Yen *et al*., 2001). The plant contains several triterpene saponins namely asiaticoside, sapogenins, asiatic acid, madecassic acid, adecassoside, vellarin, glycosides and centelloside (Duke and Ayensu, 1985; Glasby, 1991). It is rich in minerals such as calcium, magnesium, potassium, phosphorus and Aluminium (Herbert *et al*., 1994; Brinkhaus *et al*., 2000). It has been used to treat leprosy, wound, cancer, fever, syphyllis, acne, allergies (Inamdar *et al*., 1996) abscesses, headache, asthma, bronchitis, catarrh, convulsions, dysentery, eczema, gonorrhea, hypertension, jaundice, pleuritis, rheumatism, spasms, tuberculosis, ulcers and urethritis (Hausen, 1993). It has also been used as a brain tonic, psycho-physical regenerator and blood purifier (Jorge and Jorge, 2005).

The requirement of *C. asiatica* in Bangladesh is now met from the natural populations, leading to their gradual depletion. Tissue culture techniques can play an important role in the rapid multiplication of elite clones and conservation of *C. asiatica* germplasm.

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Moreover, a stable supply of the bioactive secondary products has become an utmost priority. Furthermore, there is a wide scope for application of biotechnology for the improvement of this important medicinal plant for which standardization of an efficient *in vitro* regeneration protocol is a crucial prerequisite. *In vitro* plant regeneration has been reported in *C. asiatica* through callus culture from leaf explants (Banerjee *et al*., 1999), from stem node explants (Hossain *et al*., 2000) and through somatic embryogenesis (Martin, 2004). In this paper, we describe a protocol for high frequency plant regeneration from shoot tip.

MATERIALS AND METHODS

For this investigation, shoot tips of *Centella asiatica* were collected from the Campus of the University of Rajshahi, Bangladesh. They were washed first under running tap water for 30 minutes and treated with 1% tween 80 for 10 minutes followed by repeated rinsing with sterile distilled water. Further sterilization was done under aseptic conditions in a Laminar Airflow Hood. Explants were surface sterilized with 0.1% (W/V) $HgCl_2$ for 10 minutes. Finally, the explants were washed thoroughly with autoclaved distilled water for several times to remove the traces of sterilant. The shoot tips were cut into appropriate size and cultured on MS (Murashige and Skoog, 1962) basal medium. Throughout the experiments full strength MS medium with 3% (W/V) sucrose and gelled with 0.8% (W/V) agar was used. The pH of all media was adjusted to 5.8 prior to autoclaving .The cultures were incubated in a culture room at 25 ± 2^0C with a photoperiod of 16 hour at 3000 lux light intensity provided by cool white fluorescent tubes. The basal medium was supplemented with BAP (1.0-7.0 mgl^{-1}) and NAA (0.1mgl^{-1}) of a single concentration. Rooting of shoots was achieved on full strength of MS medium supplemented with IBA at different concentrations. Well developed plantlets were removed from the culture vessels, washed gently under running tap water and planted in plastic pots containing a potting mixture of sand, soil and farmyard manure in the ratio of 1:1:1. The potted plantlets were covered by polythene sheet to maintain suitable humidity. After sufficient acclimatization, the plantlets were transplanted in the field condition, where 80% plants were survived.

RESULTS AND DISCUSSION

Proliferation of multiple shoots was observed with high frequency from shoot tips of *Centella asiatica*. This explant was capable of directly developing multiple shoots on MS medium containing different concentrations and combinations of auxin and cytokinin. The highest percentage of multiple shoot induction was 76.67% on the medium augmented with 4.0 mgl^{-1} BAP+0.1 mgl^{-1} NAA (Fig. A; Table 1) followed by 70.00% on the medium consisting of 3.0 mgl^{-1} BAP and 0.1 mgl^{-1} NAA. On the other hand, the lowest percentage of multiple shoots induction was found to be only 10.00% on the medium supplemented with 7.0 mgl^{-1} BAP and 0.1 mgl^{-1} NAA. The highest number of shoots was 10.2 ± 0.38 per explant obtained on the medium having 4.0 mgl^{-1} BAP and 0.1 mgl^{-1} NAA (Table 1; Fig. B-C) followed by 8.0 ± 0.38 shoots per explant in the medium fortified with

3.0 mgl^{-1} BAP + 0.1 mgl^{-1} NAA. On the contrary, the minimum number of shoots was 1.1±0.20 per explant on the medium supplemented with 1.0 mgl^{-1} BAP and 0.1 mgl^{-1} NAA. The induced shoots were elongated in the same medium. In the present investigation, MS medium augmented with 4.0 mgl^{-1} BAP+0.1 mgl^{-1} NAA found to be the best treatment for the highest multiple shoot induction as well as maximum number of shoots per explant. Similar results were also reported in several medicinal plants, such as, *Smilax zeylanica* L. (Sayeed Hasan and Roy, 2004); *Celastrus paniculatus* (Martin *et al*., 2006); *Heracleum candicans* (Wakhlu and Sharma, 1998); *Spilanthes mauritiana* (Bais *et al*., 2002); *Coleus blumei* (Rani *et al*., 2006).

Table 1. Effect of BAP and NAA on multiple shoot induction from shoot tip of *Centella asiatica*

Hormone conc. (mgl^{-1})		% of culture response	No. of shoots/explant (M ± SE)	Shoot length (cm) (M ± SE)
BAP	NAA			
1.0	0.1	23.33	1.1 ± 0.20	0.78 ± 0.04
2.0	0.1	36.67	3.1 ± 0.20	1.1 ± 0.07
3.0	0.1	70.00	8.0 ± 0.38	1.9 ± 0.06
4.0	**0.1**	**76.67**	**10.2 ± 0.38**	**2.3 ± 0.12**
5.0	0.1	53.33	6.2 ± 0.28	1.5 ± 0.10
6.0	0.1	33.33	4.4 ± 0.06	1.3 ± 0.07
7.0	0.1	10.00	1.93 ± 0.23	0.91 ± 0.04

Each value represents an average of 10 replicates and each experiment was repeated at least thrice, M = Mean, SE = Standard Error

Well developed shoots were isolated and cultured on MS media having different concentrations of IBA for root induction. The highest parentage of root induction was 90.00% on the MS medium augmented with 1.0 mgl^{-1} IBA (Fig. D; Table 2) followed by 73.33% on the medium with 1.5 mgl^{-1} IBA. On the other hand, the lowest percentage of rooting was 10.00% on the medium supplemented with 3.0 mgl^{-1} IBA. The highest number of roots per shoots was 10.6±0.93 from the medium augmented with 1.0 mgl^{-1} IBA followed by 8.2±0.96 roots per shoot on the medium with 1.5 mgl^{-1} IBA. On the contrary, the lowest number of roots per shoot was 0.8±0.06 in the medium fortified with 3.0 mgl^{-1} IBA. Thus, 1.0 mgl^{-1} IBA was found to be an ideal treatment for root induction. Similar results were also reported in several other medicinal plants, such as *Eclipta Alba* (Baskaran and Jayabalan, 2005); *Heracleum candicans* (Wakhlu and Sharma, 1998); *Plumbaga zeylanica* (Chaplot *et al*., 2006), *Cassia alata* (Hasan *et al*., 2008) and *Solanum trilobatum* (Jawahar *et al*., 2004).

After 40 days, well rooted plantlets were obtained. Subsequently, the plantlets were removed from the culture vessels, washed gently under running tap water and planted in pots containing a potting mixture of sand, soil and farmyard manure in the ratio of 1:1:1. The potted plantlets were covered by transparent polythene sheet to maintain suitable humidity. After sufficient acclimatization, the plantlets were transplanted in the natural

condition, where 80% plants were survived. In the present experiment, a fruitful protocol was set up through multiple shoot induction from shoot tip. This protocol can be exploited for commercial propagation and conservation of valuable medicinal plant resources.

Table 2. Effect of IBA on root induction in regenerated shoots

IBA (mgl^{-1})	% root induction	No. of roots/shoot (M ± SE)	Root length (cm) (M ± SE)
0.5	56.67	6.30 ± 0.46	4.09 ± 0.10
1.0	**90.00**	**10.6 ± 0.93**	**4.30 ± 0.03**
1.5	73.33	8.20 ± 0.96	4.20 ± 0.07
2.0	36.67	4.37 ± 0.22	4.05 ± 0.10
2.5	26.67	2.50 ± 0.23	3.70 ± 0.10
3.0	10.00	0.8 ± 0.06	2.90 ± 0.04

Each value represents an average of 10 replicates and each experiment was repeated at least thrice, M = Mean, SE = Standard Error

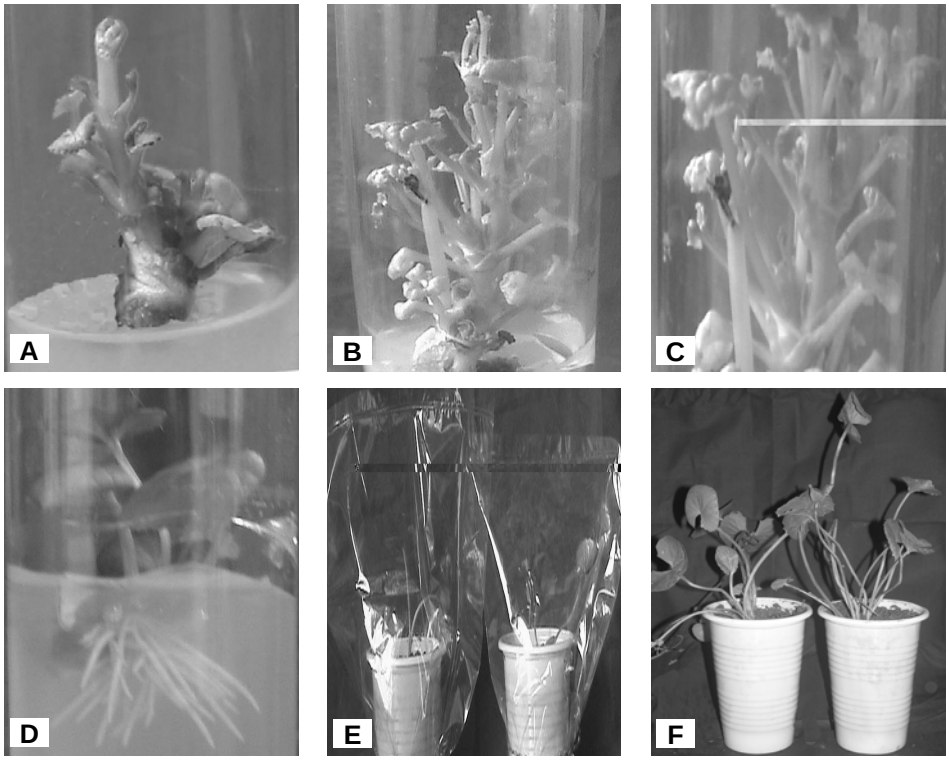

Fig. 1. Micropropagation of *Centella asiatica* using shoot tip. **A.** Multiple shoots initiation on MS+ 4.0 mg^{-1}BAP+ 0.1 mg^{-1}NAA, **B-C.** Elongation of *in vitro* shoots on the same medium, **D.** Rooting of *in vitro* shoots on MS+ 1.0 mg^{-1}IBA, **E-F**. Acclimatization of *in vitro* regenerated plants to natural condition

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