Short Communication

IN VITRO PROPAGATION OF *CENTELLA ASIATICA* (LINN.) URBAN - AN IMPORTANT MEDICINAL PLANT

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ABSTRACT

Axillary buds of *Centella asiatica* were micro-propagated in MS medium supplemented with BAP, IAA and NAA. Best response was observed on MS basal media supplemented with BAP (4 mg/l) + NAA (0.25mg/l). Every two weeks, 3-4 shoots were inoculated to a fresh medium for further shoot differentiation. Rooting of *in vitro* shoot was achieved on MS basal medium supplemented with IBA (0.4 mg/l). The rooted plants were successfully established in green house condition after hardening.

Key words: MS medium, BAP, IAA, NAA, *In vitro* rooting, hardening

INTRODUCTION

Centella asiatica (Linn.) Urban belongs to family Apiaceae. Commonly known as 'Ghortapre' in Nepali, it is widely distributed in Nepal and has great medicinal value (Chhetri and Shrestha, 2004). Its natural habitat has been markedly depleted, because of its large scale and unrestricted exploitation coupled with limited cultivation and insufficient attempts for its replacement. It has recently been listed as threatened species by the international Union for Conservation of Nature and Natural Resources (IUCN) (Pandey *et al.*, 1993) and also as an endangered species (Singh, 1989; Sharma and Kumar, 1998). *C. asiatica* is a prostrate, faintly aromatic stoloniferous, perennial, creeper herb with a glabrous stem and long petioled fleshy leaves with roots at nodes, attaining height up to 15 cm.

Centella is a reputed nerve tonic and is used for treatment of asthma, bronchitis, dropsy, elephantiasis, gastric catarrh, kidney troubles, leprosy, leucorrhoea, skin disease and urethritis (Kakkar, 1988). It shows antibacterial, antifeedant, antifilarial, antileprotic, antistress, antituberculosis activities and wound-healing properties (Chakraborty *et al.*, 1996; Srivastava *et al.*, 1997). *C. asiatica* plants are reported to contain the following glycosides: indocentelloside, brahmoside, brahminoside, asiaticoside, theankuniside and isothankuniside. Asiaticoside is useful in treatment of leprosy and tuberculosis. *Centella* leaves are rich in carotenoids, vitamins B and C. The plant shows good therapeutic effects on peptic ulcers. It is one of the components of the drug 'Geriforte' which is used for senile pruritus (Anonymous, 1992).

In recent years, there has been an increased interest in *in vitro* culture techniques which offer a viable tool for mass multiplication and germplasm conservation of rare, endangered and threatened medicinal plants (Prakash *et al.*, 1999). Tissue culture techniques also play an important role in the clonal propagation of elite clones. There has been no report to date on micro propogation of *C. asiatica* using axillary buds. However, shoot regeneration has been reported from leaf derived callus (Patra *et al.*, 1998; Banerjee *et al.*, 1999) and stem segments (Patra *et al.*, 1998) of *C. asiatica*. The present communication reports method for the *in vitro* multiplication of *C. asiatica* through axillary shoot proliferation from nodal explants followed by successful establishment of regenerated plants in soil.

MATERIALS AND METHODS

Plant material and explants preparation

Plants samples for inoculation were collected from the 'Bakhundol' area of Dhulikehl district Kavrepalanchowk where it grows around the arable lands. The plants were washed thoroughly for 45 minutes under running tap water followed by removal of leaves and roots. Nodal pieces of about 2 cm were excised from stolons. These segments were soaked in a mixture of 1% Hexinol (Agrawal Drugs Pvt. Limited) for 15 minutes. The explants were then treated with 0.1% (w/v) mercuric chloride (Ranbaxy, New Delhi, India) for 4- 6 minutes followed by five rinses with sterilized double distilled water. The nodal explants were then trimmed from both ends to about 1.0 cm prior to inoculation on culture media.

Culture medium and conditions

The culture medium used was Murashige and Skoog (1962) basal medium with 3% (w/v) sucrose and 0.8% (w/v) agar. The medium was further augmented with different concentrations of Benzyl Amino Purine (BAP) and Naphthalene Acetic Acid (NAA) in combinations (Table 1). Proliferating shoot clusters were transferred

Table 1: Effect of different concentrations of BAP and NAA on mean number of shoots, percentage response and mean shoot length

PGRs	mg/l		Mean number of shoots		Percentage of response		Mean shoot length (cm)	
BA	NAA	IAA	NAA	IAA	NAA	IAA	NAA	IAA
0.5	0.25	0.25	2.5+0.1	2.0+0.01	78.4+0.1	60.2+0.3	1.8+0.2	2.8+0.4
	0.50	0.50	2.8+0.3	2.0+0.1	86.1+0.2	80.1+0.2	1.7+0.4	2.4+0.3
	0.75	0.75	3.0+0.2	2.4+0.3	86.8+1.1	60.4+0.8	2.2+0.9	3.0+0.01
	1.00	1.00	2.4+0.7	2.1+0.3	82.3+0.6	50.6+0.4	2.5+1.0	3.2+0.4
	1.25		2.4+0.9		81.9+0.4		2.0+0.3	
1.0	0.25	0.25	5.5+0.9	3.8+0.1	80.9+0.1	90.1+0.2	2.8+1.0	2.8+0.3
	0.50	0.50	4.8+0.1	4.9+0.4	90.6+0.3	99.5+0.1	2.0+ 0.1	4.6+ 0.2
	0.75	0.75	3.6+0.4	3.2+0.3	90.9+0.2	99.8+0.5	3.6+0.04	3.8+0.1
	1.00	1.00	3.0+0.7	3.4+0.1	85.4+0.4	90.4+0.1	3.0+0.5	3.4+0.2
	1.25		2.8+1.2		80.2+0.2		2.6+0.3	
2.0	0.25	0.25	4.8+0.3	4.8+0.2	90.2+0.1	99.4+0.7	3.0+0.2	4.1+0.2
	0.50	0.50	5.0+0.1	5.8+0.01	90.6+0.3	99.2+0.2	4.5+0.1	4.6+ 0.2
	0.75	0.75	4.2+0.2	5.4+0.03	90.1+0.2	99.1+0.6	3.4+0.2	5.4+0.4
	1.00	1.00	4.0+0.1	4.9+0.1	90.4+0.4	90.6+0.3	3.2+0.5	4.5+0.01
	1.25		3.0+0.3		90.2+0.1		2.0+0.8	
3.0	0.25	0.25	4.6+0.4	3.8+0.3	90.6+0.2	90.1+0.1	2.0+0.3	3.1+0.1
	0.50	0.50	6.0+0.2	5.8+0.02	99.7+0.8	90.6+0.6	6.2+0.1	5.6+0.3
	0.75	0.75	5.2+0.01	4.0+0.4	90.6+0.6	80.2+0.4	5.8+0.01	4.2+0.1
	1.00	1.00	3.4+0.2	5.0+0.1	85.6+0.2	75.5+0.1	4.0+0.1	4.8+0.2
	1.25		2.9+0.3		85.1+0.4		3.2+0.2	
4.0	0.25	0.25	6.5+0.2	4.8+0.06	99.7+0.2	85.5+0.1	6.4+0.4	2.8+0.4
	0.50	0.50	5.8+0.1	5.4+0.02	99.2+0.6	85.6+0.6	6.3+0.01	4.9+0.2
	0.75	0.75	4.6+0.3	3.8+0.4	90.7+0.1	70.3+0.7	6.2+0.02	3.4+0.6
	1.00	1.00	2.5+0.2	3.6+0.1	85.2+0.7	70.1+0.2	6.2+0.3	3.0+0.8
	1.25		2.8+0.1		85.7+0.4		6.2+0.01	
5.0	0.25	0.25	3.4+0.01	3.5+0.1	90.6+0.3	80.1+0.2	5.0+0.1	3.2+0.1
	0.50	0.50	3.0+0.02	5.6+0.2	90.2+0.1	80.6+0.6	4.0+0.02	4.0+0.2
	0.75	0.75	2.5+0.3	5.0+0.01	90.8+0.2	75.7+0.02	5.5+0.03	3.1+0.1
	1.00	1.00	2.4+0.01	2.8+0.4	85.6+0.3	75.5+0.5	3.4+0.02	2.8+0.2
	1.25		1.9+0.02		85.6+0.4		3.8+0.2	

to MS basal and MS medium supplemented with BAP and Indole -3- Acetic Acid (IAA) for shoot elongation (Table 1). Single explant was inoculated in each test tube and closed by sterile cotton plug.

Primary shoots formed *in vitro* were sectioned into one node pieces after removing the leaves. The nodal segments containing the dormant axillary buds were cultured on MS medium supplemented with BAP and NAA for further multiplication. Subsequent subcultures were done at five week intervals.

Rooting of elongated shoots (5–6 cm) was done by subculturing on full or half-strength MS media containing different levels of Indole – 3 - Butyric Acid (IBA) (Table 2). All the cultures were incubated at 25+2°C under 16 hours photoperiod provided by cool-white fluorescent tubes (Bajaj, India).

Establishment of plants in soil

Plantlets with well developed roots were removed from culture medium and the roots were washed under running tap water to remove agar. The plantlets were transferred to plastic cups containing soil and coco peat and were covered with polythene bags to maintain the optimum relative humidity. The potted plants were maintained inside a green house by watering with tap water. After a week, the polythene bags were gradually removed over a period of 10 days and the plants were

Table 2: Effect of different concentrations of IBA on root induction from *in vitro* regenerated shoots of *C. asiatica*.

PGRs (mg/l)	Percentage response	No. of roots	Root length (cm)
IBA			
0.2	65.2+0.2	2.3+0.1	2.5+0.1
0.4	90.6+0.5	7.0+0.4	5.4+0.3
0.6	80.4+0.4	6.4+0.3	4.8+0.7
0.8	55.3+0.1	2.4+0.2	2.9+0.5
1.0	85.1+0.2	5.4+0.3	3.2+0.3

kept in the green house for another 3 weeks before transferring outside into the field.

Data analysis

The data recorded for different parameters were subjected to completely randomized design using three replications per treatment. The statistical analysis based on mean values per treatment was made using standard deviation technique for CRD.

RESULTS AND DISCUSSION

Shoot regeneration

Axillary bud culture on MS basal medium supplemented with BAP, IAA and NAA resulted in multiple shoot formation (Table: 1). The cultured buds on MS basal media showed signs of bud break only in two weeks. Media supplemented with BAP, NAA and IAA induced bud break in 10 days followed by regeneration of multiple shoots. Best response in terms of multiple shoot formation and shoot length was seen in media with BAP (4mg/l) and NAA (0.25mg/l). 6.5 shoots were produced after 3 weeks in this medium (Table 1). Maximum shoot length of 6.3 cm and mean number of shoot 5.8 were observed on MS medium with 2.0 mg/l BAP + 0.5 mg/l IAA (Table 1). In all the growth regulator combinations, multiple shoots were produced within 5 weeks of culture. The combination of BAP with NAA showed better results than BAP and IAA combination. Tiwari *et al.* (2000) also reported similar results, but Karthikeyan (2009) found effect of BAP alone better on multiple shoot regeneration.

Elongated shoots of 3-5 cm in length were separated and cultured individually on MS medium with or without an auxin (Table 2). There was no rooting on MS basal medium without auxin, but when it was supplemented with IBA moderate to profuse rooting occurred in 55–90% of the shoots. Maximum percent response (91), number of roots (7) and root length (5.4 cm) were reported on MS media containing IBA (0.4 mg/l) (Table 2). Banerjee *et al.* (1999) and Tiwari *et al.* (2000) also found promoting effect of IBA in rooting in *C. asiatica*, while IAA and low level of sucrose was reported optimum by Patra *et al.* (1998). Rooted plants were transferred from culture into pots containing soil and coco peat. The acclimatized plantlets were successfully transplanted in the field. The mortality rate was only 7 percent.

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