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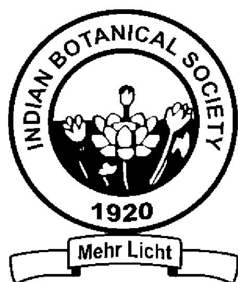

ADENINE SULPHATE ENHANCED *IN VITRO* SHOOT REGENERATION IN *CENTELLA ASIATICA* (L.) URBAN

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A protocol for multiple shoot regeneration in *Centella asiatica*, a medicinally valuable plant, is developed. Supplementation of MS medium with BAP (1.0 mg/l) and adenine sulphate (0.072 g/l) enhances shoot multiplication from leaf explants up to 11shoot buds per explant and 14-15 leaves per shoot with petiole length of about 7.0 cm as against 3.38 shoots per explant with 4.25 leaves per shoot reported so far. Increased number of leaves may enhance the content of bioactive metabolites especially asiaticoside to considerable extent reducing overexploitation of this valuable herb.

Key words: adenine sulphate, *Centella asiatica*, shoot multiplication

Centella asiatica (L.) Urban is mandookparni of ayurveda and gotukola of international commerce. It is a highly important source of drugs for both modern and traditional systems of medicine. It has been widely recommended for wound healing, treatment of skin lesions, leprosy, ulcers, eczema and psoriasis (Kirtikar and Basu 1933). It is one of the medicinal plants that have much prized OTC (Over The Counter) drugs in developed countries (Tiwari *et al.* 2001). It is used as nervine tonic for improving memory, as a diuretic, alternative, used in skin diseases, tuberculosis and dysentery (Jain and Defillips 1991).

The plant contains glycosides viz., brahmoside, indocentelloside, asiaticoside, theankuniside, isotheankuniside, madecassoside and brahminoside (Dutta *et al.* 2001, Tiwari *et al.* 2001). Asiaticoside, a trisaccharide triterpene, has been identified as the most active compound in the plant responsible for its pharmaceutical value. *Centella asiatica* has made a significant impact in global medicinal herbs so its demand in national and international herbal market is increasing day by day. It has been estimated that on an average about 700 tonnes of plant material is required per year by various pharmacological and pharmaceutical industries (Chandrika *et al.* 2002). Over exploitation of this plant by pharmaceuticals led this plant under threat of extinction. Thus increasing medical interest underlines the necessity of proper domestication and conservation of medicinal plants as potential source of medicine (Franz 1993, King 1995). Tissue culture is one such method to obtain uniformly, vigorous plants for production of therapeutic compounds (Hirai *et al.* 1997). So the present studies have been undertaken to device an optimal condition for propagation of *Centella* plant under *in vitro* conditions.

MATERIAL AND METHODS

Plants of *Centella asiatica* were collected from F.R.I., Dehradun. Young leaves as well as petiole explants were collected from field grown plants. These explants were initially washed with a detergent solution (Tween-20) for 2-3 minutes, followed by thorough washing under tap water for 10-15 minutes. These explants were surface sterilized with 0.01% $HgCl_2$ for 20 seconds, followed by 70% alcohol and then washed with double distilled water. The sterilized leaf and petiole were dissected into about 1.0 cm explants and inoculated one explant per culture tube on

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MS medium (1962) containing different concentrations of cytokinin (0.5-3.0 mg/l of BAP/ Kn) and auxin (0.01-0.1 mg/l NAA). Incubation was carried out under 1400 lux intensity with a photoperiod of 16/ 8 light/ dark cycles and 25 ± 2^0C temperature. Callus induced from leaf explants on MS medium with BAP (2.0 mg/l) and NAA (0.1 mg/l) was chosen for further studies due to its better quality and quick response.

RESULTS

Caulogenesis: Induction of shoot buds from callus occurred on the same medium that is on MS medium with BAP (2.0 mg/l) and NAA (0.1 mg/l) after two subcultures with very low frequency of shoot formation (Fig.1A). Shoots produced on this medium were weaker and longer than those initiated on MS medium with BAP (1.0 mg/l) (Fig.1B), though with low frequency as in the former. Shoots initiated on MS medium with BAP (1.0 mg/l) were then transferred on MS medium with BAP (1.0 mg/ l) and adenine sulphate (24-96 mg/l). Frequency of shoot initiation increased with increase in concentration of adenine sulphate in MS medium (Fig.1C, D, E). But this increase was observed up to 72 mg/l of adenine sulphate (Fig.1E), beyond that (96 mg/l) no shoots initiated in the callus. Best results in terms of early response, shoots produced/ flask, number of leaves/ shoot and petiole length was observed in MS medium with BAP (1.0 mg/l) and Adenine sulphate (72 mg/l) (Table-1).

Rhizogenesis: Shoots were allowed to grow up to 2-3 cms. For rooting, healthy shoots were excised from flasks and sub cultured on MS +NAA (1.0-5.0 mg/l). Rooting from shoots occurred in all concentrations of NAA used, but MS +NAA (5.0 mg/l) gave best results (Table-2). In this medium prostrate stems showed rooting at nodes also.

Hardening: After 3 weeks plantlets (Fig.1F) were transferred in 500 g glass jars (with poly-propylene caps), half filled with sterilized sand: soil (1:1) or peat: soilrite (1:1) mixtures. These mixtures were moistened with about 20 ml autoclaved and cooled $^1/_2$ MS liquid basal medium with out any sucrose and were maintained in the culture incubation chamber. Sterile peat: soilrite (1:1) (Fig.1G) mixture proved to be better medium for hardening of plants rather than sand: soil mixture. After 20-25 days healthy plantlets were shifted to potting mixture of leaf litter: sand: moss (1:1:1) (Fig.1H) and finally to green house Sharma (2007). However, further work is needed to improve percent survival of plantlets when transferred to natural field conditions.

DISCUSSION AND CONCLUSION

Rao *et al.* (1999) reported stolon and stem to be the best for callus induction followed by leaf explants, whereas our studies recorded leaf explants as the best responding explant. Sangeeta *et al.* (2003) developed a rapid clonal propagation method for *C. asiatica* on MS medium with BA alone or with NAA and Kn. In their report optimum number of shoots (3.38) with optimum number of leaves/ shoot (4.25) was attained on MS medium with BA (4.0 mg/ l) and NAA (0.1 mg/l). Whereas Tiwari *et al.* (2001) described *in vitro* clonal propagation by enhanced axillary bud proliferation from nodes of *C. asiatica.* They reported supplementation of 22.2µM BA and 2.68µM NAA in MS medium to induce optimum frequency (91%) of shoot formation as well as shoot number/ node (4-5) in *C. asiatica.* In the present studies shoot initiation was maximum in MS medium with BAP (1.0 mg/l or 4.44µM) and adenine sulphate (0.072 g/l) with 11 shoot buds/ explant and 14-15 leaves/ shoot with an average petiole length of 7.0 cm. Similar results have been shown by Ramesh *et al.* (2006) in *Bacopa* with 0.060 g/l adenine sulphate and by Debnath *et al.* (2000)

Table 1: Shoot regeneration from callus on MS medium supplemented with different plant growth regulators

S. No.	MS medium +growth hormone	No. of shoots/ flask (± SD)	No. of leaves/ shoot (± SD)	Length of petiole (cm ± SD)
1.	BAP (1.0 mg/l)	1.33±0.57	3.0±0.70	3.33±0.57
2.	BAP(2.0 mg/l)+ NAA(0.1mg/l)	1.33±0.42	2.0±0.51	2.56±0.60
3.	BAP (1.0 mg/l)+ AS (0.024 g/l)	2.33±0.61	4.0±0.33	4.26±0.64
4.	BAP (1.0 mg/l)+ AS (0.048 g/l)	3.33±0.57	8.0±0.55	4.06±0.12
5.	BAP (1.0 mg/l)+ AS (0.072 g/l)	11.2±1.3	14.6±1.8	7.0±0.14
6.	BAP (1.0 mg/l)+ AS (0.096 g/l)	No response	No response	No response

AS-Adenine Sulphate, BAP-Benzyl Amino Purine, NAA- Naphthalene Acetic Acid, ± SD- Standard Deviation

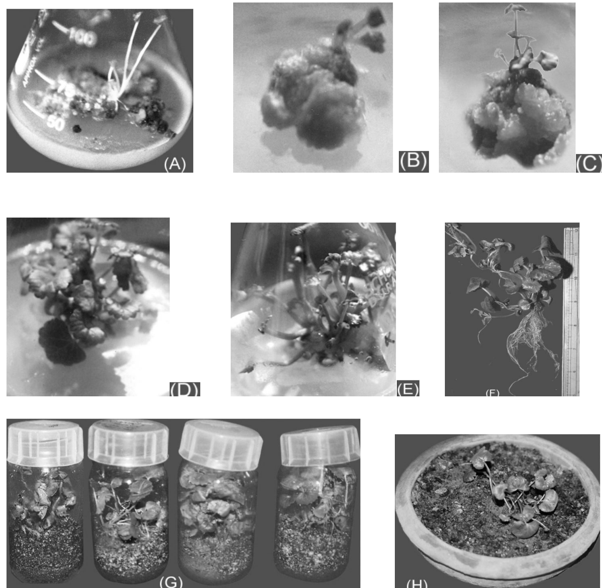

Figure-1 A-H: **AS enhanced *In Vitro* shoot regeneration in *Centella asiatica* (L.) Urban**

A- Shoot regeneration in MS + BAP (2.0 mg/l) + NAA (0.1 mg/l), B- Shoot regeneration in MS + BAP (1.0 mg/ l), C- Shoot regeneration in MS + BAP (1.0 mg/l) + AS (0.024 g/l), D- Shoot regeneration in MS + BAP (1.0 mg/l) + AS (0.048 g/l), E- Shoot regeneration in MS + BAP (1.0 mg/l) + AS (0.072 g/l), F- Regenerated *Centella asiatica* plant., G and H- Hardening.

in peach with 10 mg/l adenine sulphate. Basically, adenine sulphate being sulphonated form of a nucleotide base has been proposed to induce high frequency shoot organogenesis in *Cichorium intybus* L. callus also (Nandgopal and Ranjitha Kumari 2006). In the present studies along with benzyl amino purine, sulphate of a purine probably provides an easy to metabolize form towards protein synthesis to *C. asiatica* calli through sulphur assimilation.

Table 2: Effect of Auxin on rooting from in vitro raised shoots in MS media after 4 weeks of culture

S. No.	MS medium + growth hormone	Percent rooting	No. of roots per explant	Average root length (cm)
1.	NAA(1.0 mg/l)	62 %	4-5	1.5
2.	NAA (2.0 mg/l)	60 %	2-3	2.5
3.	NAA (3.0 mg/l)	72 %	6-7	3.0
4.	NAA (4.0 mg/l)	80 %	8-10	3.9
5.	NAA (5.0 mg/l)	85 %	10-12	4.2

Leaves of *Centella* are reported to be the best source of asiaticoside. Present study proves supplementation of MS medium with BAP (1.0 mg/l) and adenine sulphate (72 mg/l) to be optimum for multiple shoot regeneration with increased number of leaves from callus of *Centella.* Results obtained from present studies are an improvement over previous reports. Thus, multiplying plants by this protocol is much significant to meet the growing need of plant for Asiaticoside yield with out destroying the natural green cover.

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REFERENCES

Chandrika K, Lakshmipathy R, Gowda B, Balakrishna AN, Rajanna MD & Bagyaraj DJ 2002 Response of *Centella asiatica* (L.) Urb. To VA mycorrhizal inoculation. *J Soil Biol Ecol* **22**(1&2) 35-39.

Debnath RK, Roy SK, Ahmed G & Hossain M 2000 Micropropagation of Patal (*Trichsanthes dioica* Roxb.) from nodal segment and shoot tip. *Plant Tissue Cult* **10**(2) 125-130.

Dutta D, Sen P & Deka PC 2001 Isolation of active ingredients from *C. asiatica. Annals of Agri Bio Research* **6**(1) 15-17.

Franz C 1993 Domestication of wild growing medicinal plants. *Plant Res Dev* **37** 99-111.

Hirai G, Kasai N & Harada T 1997 Somatic embryogenesis in mature zygotic embryo of *Glehnia littoralis. Plant Cell Tissue*

and Organ Culture 75-180.

Jain SK & Defillips R 1991 Asclepiadaceae in Algonae, M.I. (ed.), Medicinal Plants of India. Reference Publication Inc., Michigan, U.S.A. **1**, 144-152.

King SR 1995 The past, present and future importance of plant-derived medicines (Economic Botany). *Amer J Bot* **82**(6) 66.

Kirtikar KR & Basu BD 1933 Indian medicinal plants, **1**(2) edition L. M. Basu, Allahabad, 440.

Murashige T & Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plantarum* **15** 473-497.

Nandgopal S & Ranjitha Kumari 2006 Adenine Sulphate induced high frequency shoot organogenesis in callus and *in vitro* flowering of *Cichorium intybus* L. cv. Focus- a potent medicinal plant. *Acta Agriculturae Slovenica* **87**(2) 415-425.

Ramesh M, Sarvankumar RM & Pandian S 2006 Benzyl amino purine and adenine sulphate induced rapid multiple shoot and root induction from nodal explants of Brahmi [*B. monnnieri* (Linn.) Penn.]. *Natural Product Radiance* **5** 44-51.

Rao KP, Rao SS & M. Sadanandam 1999 Tissue culture studies of *C. asiatica. Indian J. of Pharm. Sci.,* **61**(6): 392-395.

Sangeeta N & Buragohain A K 2003 *In vitro* method for propagation of *C. asiatica* (L) Urban by shoot tip culture. *J of Plant Biochem And Biotech* **12**(2) 167-169.

Sharma S 2007 *In vivo* and *in vitro* biochemical studies in some medicinal plants. Ph.D. Thesis, C.C.S. University, Meerut (India).

Tiwari KN, Sharma NC Tiwari V & Singh BD 2001 Micropropagation of *Centella asiatica* (L.) Urban. *Plant Cell Tissue and Organ Culture* **63**(3) 179-185.