BCSIR

Available online at www.banglajol.info

Bangladesh J. Sci. Ind. Res. **45(1)**, 69-74, 2010

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

E-mail: bjsir07@gmail.com

In Vitro Mass Propagation of *Heliotropium indicum* L., using Apical and Axillary Bud Explants

A. K. M. Sayeed Hassan*, Nadira Begum, Miskat Ara Akhter Jahan and Rahima Khatun

Biological Research Division, BCSIR Laboratories, Dhaka-1205, Bangladesh.

Abstract

A consequency was obtained for mass propagation of a valuable ayurvedic medicinal herb, *Heliotropium indicum* Linn. (Boraginaceae) through *in vitro* culture. Apical and axillary buds of young sprouts from selected plants were used as explants. Best shoot induction was observed on MS basal medium supplemented with 0.5 mg/l BAP + 0.1 mg/l GA_3, in which 92% of the axillary buds explants produced 12 shoots per culture. Repeated subcultures in the same medium, resulted rapid shoot multiplication with 18 shoots per culture. *In vitro* raised shoots rooted on half strength MS medium with 0.5 mg/l IBA. For acclimatization and transplantation, the plantlets in the rooting culture tubes were kept in normal room temperature for 7 days before transplanting in pots where plantlets were reared for three weeks. The survival rate of regenerated plantlets was 85%.

Key words: *Heliotropium indicum,* Medicinal plant, Shoot proliferation, Micropropagation, Acclimatization**.**

Introduction

Heliotropium indicum Linn. commonly known as 'Hatisur' belongs to the family - Boraginaceae, a small fragrant plant with rough, ovate-oblong leaves and small white flowers in compact curved cymes, grows in waste places and sides of ditches in all parts of the country (Ghani, 1998). Leaf juice is used in eye diseases and is applied locally to ulcers, sores, wounds, gum boils, skin affections, stings of insects. Leaf extract is used in rheumatism and also possesses antineoplastic property. Plant possesses emollient, vulnerary and diuretic properties. Decoction of leaves and young shoots is used against urticaria, ringworm, rheumatism, gonorrhoea, pharyngitis and tonsillitis. Root is used for the cure of night blindness and aphrodisiac (Ghani, 1998). Seeds are masticated and swallowed as stomachic. The stems and leaves contain tannin with an uncertain alkaloidal properties. The leaves dye an impermanent black (Anonymous, 1959).

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 (Ajithkumar and Seeni, 1998; Prakash *et. al.*, 1999). Commercial exploitation and elimination of natural habits consequent to urbanization has led to gradual extinction of several medicinal plants. Micropropagation is an effective approach to conserve such germplasm. Further, genetic improvement is another approach to augment drug-yielding capacity of the plant (Tejavathi and Shailaja, 1999). *In vitro* propagation has proven as a potential technology for mass scale production of medicinal plant species (Lui and Li, 2001; Wawrosch *et. al*., 2001; Martin, 2002 and 2003; Azad *et. al.*, 2005; Faisal *et. al*., 2003; Hassan and Roy, 2005). It is important, therefore to develop an efficient micropropagation technique for *Heliotropium indicum* Linn. for rapid disseminate with superior identified clones. There have been few reports to date on micropropagation of *Heliotropium indicum* Linn. using apical and axillary bud explants. There is no report on the establishment of a micropropagation protocol for *Heliotropium indicum* Linn. in Bangladesh. The present study was therefore undertaken to develop a protocol for mass clonal propagation of this important medicinal herb through *in vitro* culture.

Materials and Methods

The experiment was conducted at Biological Research Division in Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. Healthy and profusely growing vine of *Heliotropium indicum* Linn. was collected from research field of BCSIR Campus, Dhaka and used as source of explants. Shoot tips and nodal explants with a single axil-

* Corresponding author: E-mail: Aksayeed sc @ gmail.com

lary bud were used for this purpose. The explants were washed thoroughly under running tap water, pre-soaked in liquid detergent for about 30 min, wiped with cotton and dipped in 70% (v/v) ethanol for 1 min. They were then surface-sterilized with 0.1% (w/v) mercuric chloride for 5 min, followed by five times rinse with sterile distilled water under laminar air flow cabinet. The surface-sterilized explants were sized to 1-1.5 cm length containing a single node with an axillary bud or a shoot tip with an apical bud. The explants were placed vertically on the culture medium. The new shoots induced from the *in vitro* cultures were further used as an explants for adventitious shoot regeneration.

MS (Murashige and Skoog, 1962) basal medium was used for shoot proliferation and adventitious shoot regeneration and half strength MS was used for *in vitro* root induction. All media were supplemented with 30 g/l sucrose, 7 g/l agar (Difco) and dispensed into 15x150 mm culture tubes and 250 ml conical flasks. The pH of the media was adjusted to 5.8 before autoclaving at 121^{O}C for 20 min. The cultures were incubated for a 16 h photoperiod at 24 ± 2^{O}C under a fluorescent light.

Shoot proliferation from apical and axillary bud explants was obtained in two separate sets of experiments. In the first experiment 0-2.0 mg/l BAP and 0-2.0 mg/l Kn were incorporated into MS media to select the best cytokinin for response of shoot induction. In the second set, combination of BAP (0-2.0 mg/l) with NAA (0.1-0.5 mg/l), BAP (0-2.0 mg/l) with IAA (0.1-0.5 mg/l) and BAP (0.5 mg/l) with GA_3 (0.1mg/l) were assessed for shoot multiplication. Number of new shoot proliferation of each culture was recorded after every week of inoculation.

For *in vitro* rooting, individual shoots (3-5 cm) were excised from the proliferated shoot cultures and implanted onto half strength MS media with different concentrations and combinations of NAA, IBA and IAA.

The rooted plants were taken out from the culture tubes, washed to remove agar gel adhered to the roots and transplanted to plastic pots with soil and compost (1: 1) for hardening. The plantlets were kept in a polychamber at 80% relative humidity, 32 ± 2^{O}C under a 12 h photoperiod for acclimation. Established plants were transplanted in earthen pots under natural conditions and the survival rate was recorded.

Results and Discussion

Apical and axillary bud explants of *Heliotropium indicum* Linn. were cultured on MS media supplemented with BAP alone and various concentration of BAP with NAA, IAA or GA_3 for multiple shoot regeneration. The explants were found to be swollen and they produced two to three shoots within three-four weeks after inoculation (Fig.

1a & b) on MS media containing BAP alone but the number of shoots increased up to 12 when the explants were cultured in MS with 0.5 mg/l BAP + 0.1 mg/l GA_3 (Fig. 1c). Combinations of BAP with NAA or IAA were not found to be suitable than BAP with GA_3 for shoot induction (Table I) and combinations of Kn alone and Kn with NAA or IAA were also not found to be suitable for shoot induction (Data not shown). Both the explants responded in the same medium but highest numbers of micro shoots were observed to be induced from axillary buds explants (Fig.1d). Newly initiated shoots were separated and sub cultured repeatedly in fresh MS with 0.5 mg/l BAP + 0.1 mg/l GA_3, where the number

of shoots increased up to 18.4 ± 1.0 per culture within ten to twelve weeks (Table I, Fig. 1d). Nearly same response of microshoot were obtained in MS medium fortified with 0.5 mg/l BAP + 0.1 mg/l GA_3 and 0.5 mg/l BAP alone but healthier shoots were found in the former medium content. Kumer and Rao (2007) reported that the highest number of shoots was yielded after 30 days of culture in MS medium supplemented with 1.0 mg/l Kn, 0.5 mg/l BAP and 0.05 mg/l IAA. The cluster of proliferated shoots elongation were observed simultaneously on the same medium. In different medicinal plant, it was also observed that multiple shoots were found by using different concentration of cytokinin with auxins by other researchers (Datta *et al.*, 2003; Gawde and Paratkar, 2004; Baskaran and Jayabalan, 2005; Husain and Anis, 2006; Han *et al.*, 2007; Usha *et al.*, 2007; Hassan, 2008; Afroz *et al.*, 2008).

85.2% regenerated shoots rooted (Fig. 1e) when cultured individually on root induction medium consisted of half-strength MS medium with 0.5 mg/l IBA (Table II). Use of auxins alone or its combination for rooting was also reported by different authors (Sahoo and Chand, 1998; Ajithkumar and Seeni, 1998; Rai, 2002; Sivakumar and Krishnamurthy, 2000; Hassan and Roy, 2004; Rahman *et al.*, 2006; Baksha *et al.*, 2007; Hassan *et al*., 2008).

After four weeks the rooted shoots were transferred to pots. None of the plantlets were survived when transferred directly from rooting medium to the pot under natural conditions. About 85 percent of the transplanted plants of *Heliotropicum indicum* Linn. survived if the plants were kept in the rooting culture tubes in normal room temperature for seven days before transplantation in pots and reared for three weeks. The plantlets were reared under semi-controlled temperature ($30\pm2^{O}C$) and light (2000 lux) in a chamber with 80 percent humidity. During this period of acclimation shoots elongated, leaves expanded and turned deep green and healthier (Fig. 1f and g).

After three weeks, plants were transferred to an open place and gradually acclimated to outdoor conditions, where 85 percent plants were survived. The technique described here appears to be readily adaptable for large scale clonal propagation and plantation for sustainable use in the industry. Moreover, by standardizing the protocols for clonal propagation of selected elite plants, it is possible to achieve a tenfold increase in the products per unit area of cultivation (Hassan and Roy, 2005). Clonally propagated plants would also have identical phytochemical profiles (Roja and Heble, 1993).

Table II. Effect of auxin and its combination on root induction in regenerated shoots of *Heliotropium indicum* Linn. on half strength MS

Data were recorded after four weeks of culture. Results are mean ± SE of 15 replications.

Likewise it could be possible to propagate important medicinal plants for cultivation and sustainable use and consequently to conserve them from their extinction.

Acknowledgement

The authors are indebted to Professor Dr. Shyamal K. Roy, Department of Botany, Jahangirnagar University, Savar, Dhaka. Grateful acknowledgement is also due to the Director, BCSIR Laboratories, Dhaka and Professor Elias Khan, Principal, Savar College, Savar, Dhaka for their kind permission, sincere suggestions and moral support during research work.

References

Afroz F., Hassan A. K. M. S., Bari L.S., Munshi J. L., Jahan M. A. A. and Khatun R. (2008) *In vitro* regeneration of *Vitex negundo* L., a woody valuable medicinal plant through high frequency axillary shoot proliferation. *Bangladesh J. Sci.Ind. Res*. **43**(3): 345-352.

Ajithkumar D. and Seeni S. (1998) Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Aegle marmelos* (L) Corr., A Medicinal Tree. *Plant Cell Rep.* **17**: 422-426.

Anonymous (1959) The Wealth of India: Raw Materials.

CSIR, New Delhi, India.Vol. V: H-K, pp 29-31.

Azad M. A. K., Yokota S., Ohkubo T., Andoh Y., Yahara S. and Yoshizawa N. (2005) *In vitro* regeneration of the medicinal woody plant *Phellodendron amurense* Rupr. Through excised leaves. *Plant Cell Tiss. Orga. Cult*. **80**: 43-50.

Baskaran P. and Jayabalan N. (2005) An efficient micropropagation system for Eclipta alba - a valuable medicinal herb. *In Vitro Cell. Dev. Biol. Plant.* **41**: 532-539.

Baksha R., Jahan M. A. A., Khatun R. and Munshi J. L. (2007) *In vitro* rapid clonal propagation of *Rauvolfia serpentina* (Linn.) Benth. *Bangladesh J. Sci Ind. Res.* **42**(1): 37-44.

Datta A., Narula A., Bansal K. C. and Srivastava P. S. (2003) Enhanced alkaloid production in callus culture of *Heliotropicum indicum* L. and regeneration of plantlets. *Journal of plant Biochemistry and Biotechnology*. **12**(2): 139-142.

Faisal M., Ahmad N. and Anis M. (2003) Shoot multiplication in *Rauvolfia tetraphylla* L. using thidiazuron. *Plant Cell Tiss. Organ. Cult.* **80**: 187-190.

Gawde A.J. and Paratkar G.T. (2004) Micropropagation of *Eclipta alba* Hassk - An approach to shorten the protocol. *Indian Journal of Biotech.* **3**(1): 128-1323.

Ghani A. (1998) Medicinal Plants of Bangladesh with Chemical Constituents and Uses. 2nd Ed. (Asiatic military press, Dhaka, 1000) pp 373.

Han L., Guo S. and Chang M. (2007) Tissue culture of *Eclipta prostrata* L. *Chinese Pharmaceutical Journal*. **42**(2): 94-98.

Hassan A. K. M. S. and Roy S. K. (2004) Micropropagation of *Smilax zeylanica* L., a perennial climbing medicinal shrub, through axillary shoot proliferation. *Bangladesh J. Life Sci.* **16**(1): 33-39.

Hassan A. K. M. S. and Roy S. K. (2005) Micropropagation of *Gloriosa superba* L. through high frequency shoot proliferation. *Plant Tissue Cult. and Biotech.* **15**(1): 67-74.

Hassan A. K. M. S. (2008) *In vitro* Regeneration of a woody medicinal plant, *Pterocarpus santalinus* Linn. f., through apical and axillary shoot proliferation. *Bangladesh J. Life Sci.* **20**(1): 29-36.

Hassan A. K. M. S., Afroz F., Bari L. S., Munshi J. L., Jahan M. A. A. and Khatun R. (2008) Callus induction and high frequency regeneration of plantlets of *Scoparia dulcis* L., a perennial medicinal herb, through axillary shoot proliferation. *Plant Tissue Cult. and Biotech.* **18**(1): 75-83.

Husain M. K. and Anis M. (2006) Rapid *in vitro* propagation of *Eclipta alba* (L.) Hassk. through high frequency axillary shoot proliferation. *Acta Physiologiae Plantarum*. **28**(4): 325-330.

Kumer M. S. and Rao M. V. (2007) *In vitro* micropropagation of *Heliotropicum indicum* Linn.- an ayurvedic herb. *Indian Journal of Biotechnology.* **6**: 245-249.

Lui Z. and Li Z. (2001) Micropropagation of *Camptotheca acuminata* Decaisne. from axillary buds, shoot tips and seed embryos in tissue culture system. *In Vitro Cell Dev. Biol. Plant.* **37**: 84-88.

Martin K. P. (2002) Rapid propagation of *Holostema adakodien* Schult. a rare medicinal plant, through axillary bud multiplication and indirect organogenesis. *Plant Cell Rep.* **21**: 112-117.

Martin K. P. (2003) Plant regeneration through somatic embryogenesis on *Holostema ada-kodien* Schult. a rare medicinal plant. *Plant Cell Tiss. Orga. Cult.* 72: 79-82.

Murashige T. and Skoog F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* **15**: 473-479.

Prakash E., Sha Valli Khan P. S., Sairam Reddy P. and Rao K. R. (1999) Regeneration of plants from seed-derived callus of *Hybanthus enneaspermus* L. Muell., a rare ethnobotanical herb. *Plant Cell Rep.* **18**: 873-878.

Rahman S. M. R., Afroz F., Sultana K., Sen, P. K. and Ali M. R. (2006) Effect of growth regulators and state of medium on micropropagation of *Adhatoda vasica* (Nees.). *Khulna University Studies*, Special Issue (1st Research Cell Conference): 55-59.

Rai V. R. (2002) Rapid clonal propagation of *Nothapodytes foetida* (Weight) Sleumer - a threatened medicinal tree. *In Vitro Cell Dev. Biol.Plant*, **38**: 347-351.

Roja G. and Heble M.R. (1993) The quinoline alkaloids camptothecin and 9-methoxy camptothecine from tissue culture and mature trees of *Nothapodytes foetida. Phytochemistry.* **36**: 65-66.

Sahoo Y. and Chand P.K. (1998) Micropropagation of *Vitex nigundo* L., a woody aromatic medicinal shrub, through high frequency axillary shoot proliferation. *Plant Cell Rep*. **18**: 301-307.

Sivakumar G. and Krishnamurthy K. V. (2000) Micropropagation of *Gloriosa superba* L.- an endangered species of Asia and Africa. *Current Scence.* **78**(1): 30-32.

Tejavathi D. H. and Shailaja K. S. (1999) Regeneration of plants from the cultures of *Bacopa monnieri* (L) *Pennell. Phytomorphology.* **49**(4): 447-452.

Usha P. K., Benjamin S., Mohanan K. V. and Raghu A. V. (2007) An efficient micropropagation system for *Vitex negundo* L., an important woody aromatic medicinal plant, through shoot tip culture. *Research journal of Botany.* **2**(2): 102-107.

Wawrosch C., Malla R. R. and Kopp B. (2001) Clonal propagation of *Lilium nepalense* D. Don, a threatened medicinal plants of Nepal. *Plant Cell Rep.* **10**: 457-460.

Received : March 30, 2009;
Accepted : July 27, 2009