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E-mail: bjsir07@gmail.com

In Vitro Clonal Propagation of *Scoparia dulcis* L., a Perennial Medicinal Herb

A. K. M. Sayeed Hassan*, Laila Shamroze Bari, Rebeka Sultana, Nadira Begum and Rahima Khatun

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

Abstract

An efficient protocol was established for *in vitro* clonal propagation of the perennial medicinal herb *Scoparia dulcis* L. (Family. Scrophulariaceae) 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 0.1 mg/l BAP, in which 94% of the explants produced 12 shoots per culture. Repeated subcultures in the same medium, resulted rapid shoot multiplication with 16 shoots per culture. The half strength MS medium with 0.5 mg/l IBA +0.5 mg/l NAA the highest percentage (85.20) and maximum number (13.40) of roots were initiated within four weeks of culture. 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: *Scoparia dulcis*, Medicinal plant, Shoot proliferation, Micropropagation, Acclimatization, IAA (indoleacetic acid), IBA(indolebutanoic acid), NAA(α-naphthaleneacetic acid), BAP(benzylamino purine)

Introduction

Scoparia dulcis L. commonly known as 'chinigura' (F-Scrophulariaceae) a small erect, slender, rigid, perennial herb with three serrate-margined, ovate-elliptic leaves at each node, small white, axillary, solitary flowers and small coriander-like fruits, grows as a common weed in all areas of Bangladesh (Ghani, 1998). Ten species of Scoparia were noted from Argentina (Escandon *et al.* 2005). Among them *Scoparia dulcis* is abundandly distributed in many tropical countries like India (Anonymous, 1972) and Bangladesh. Leaf of *Scoparia dulcis* is used as a cure for gastric ulcer and physical weakness. Infusion of leaves is used in fever, cough, bronchitis, diarrhoea and dysentery and as a diuretic and gargle for toothache. Decoction of the leaves is useful in curing gavel and kidney problems. The plant is also used in diabetes and to stop bleeding (Ghani, 1998). All parts of the plant are useful as emetic. An infusion of seeds obtained by soaking them in water overnight is a cold drink.

The plant is used as cattle fodder. An antidiabetic compound, amellin has been reported in the leaf and stem of the fresh green plant. Oral administration of amellin is reported to relieve symptoms of glycosuria, reduce hyperglycaemia and increase RBC count. It has also been found to be helpful in anaemia, albuminuria, ketonuria, retinitis and other complication associated with diabetes mellitus. Unlike insulin, amellin does not cause blood suger levels to drop below normal and reduction of both blood and urine sugar occur gradually. Amellin is reported to raise the lowered alkali reserves in diabetics and reduce iron content of serum and of acetone bodies in blood (Anonymous, 1972).

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, 2003; Azad *et al*. 2005; Faisal *et al.* 2003; Hassan and Roy, 2005). Therefore it is important to develop an efficient micropropagation technique for *Scoparia dulcis* for rapid disseminate superior clones once they are identified. Tissue culture technique can play an important role in the clonal propagation of elite clones and

* Corresponding Author: E-mail:aksayeedsc@gmail.com

germplasm conservation of this medicinal herb. There have been few reports to date on micropropagation of *Scoparia dulcis* using shoot tips and nodal explants. However, in Bangladesh, there is only one report on the establishment of a micropropagation protocol through callus culture for *Scoparia dulcis*. The present study was, therefore, undertaken to develop a protocol for mass clonal propagation by using shoot tips and nodal explants of this important medicinal herb through *in vitro* culture.

Materials and Method

The experiment was conducted at Biological Research Division in Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. Healthy and profusely growing vine of *Scoparia dulcis* L. was collected from BCSIR campus, Dhaka and used as source of explants. Shoot tips and nodal explants with a single axillary 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 rinses with sterile distilled water in front of a 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* rooting. All the 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 shoot tips and nodal explants was obtained in two separate sets of experiments. In the first experiment 0-2.5 mg/l BAP and 0-2.5 mg/l Kn were incorporated into MS to select the best cytokinin for the response of shoot induction. In the second set, combination of BAP (0.1-0.3 mg/l)-NAA (0.05-0.1 mg/l) and BAP(0.1-0.3 mg/l)-IAA (0.05-0.1 mg/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 into half strength MS with different concentrations and combinations of NAA, IBA and IAA.

The rooted plantlets 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.

Table I. Effect of growth regulators in MS on morphogenic response of *Scoparia dulcis* shoot tips and nodal segments

Growth regulators (mg/l)			Shoot tips		Nodal segments	
BAP	NAA	IAA	Explants forming shoots (%)	Mean No. of shoot/explant	Explants forming shoots (%)	Mean No. of shoot/explant
Growth	regulator	free	-	-	-	-
0.05			73.2±1.36	03.2± 0.52	76.2±2.42	06.2±0.76
0.1			94.4±1.45	12.4±0.60	96.2±0.52	16.4±0.82
0.2			83.4±1.21	06.0± 0.63	88.4±1.45	08.2±0.59
0.3			68.8±2.35	04.2± 0.43	72.0±1.35	06.0±0.63
0.1	0.05		81.6±1.40	09.0± 0.74	83.4±1.21	12.4±0.60
0.2	0.1		76.2±2.42	06.2± 0.76	79.2±1.86	06.2±0.76
0.3	0.1		71.4±1.40	04.2± 0.43	74.4±1.48	04.4±0.45
0.1		0.05	78.2±2.021	09.0±0.74)	81.6±1.40	12.2±0.76
0.2		0.1	72.0±1.35	04.4±0.45	76.2±2.42	06.2±0.76
0.3		0.1	68.8±2.35	02.4±0.45	72.0±1.35	03.6±0.45

Results are mean ± SE of three experiments with 15 replications.

Results and Discussion

Shoot tips and nodal explants of *Scoparia dulcis* L. were cultured on MS media supplemented with various concentration of BAP alone and BAP with NAA or IAA for shoot regeneration (Fig. 1a). The explants were found to be swollen and they produced four to five shoots within three-four weeks after inoculation on MS containing 0.1 mg/l BAP (Table I, Fig. 1b). Both the explants responded in the same medium but highest numbers of micro shoots were observed to be induced from nodal explants. Combinations of BAP with NAA and IAA were not found to be suitable for shoot induction (Table I). Newly initiated shoots were separated and sub cultured repeatedly in fresh MS with 0.1mg/l BAP, where the number of shoots increased up to 16.4 per culture (Table I, Fig 1c). Hassan *et al.* (2008) reported that green compact

Fig 1: *In vitro* regeneration of *Scoparia dulcis* from shoot tip and nodal explants.

(a) Inoculation of explants on regeneration medium.
(b) Shoot multiplication on MS + 0.1 mg/l BAP after eight weeks of culture.
(c) Development and multiplication of shoots on MS + 0.1 mg/l BAP after twelve weeks of culture.
(d) Rooting of *in vitro* regenerated shoots cultured on half strength MS + 0.5 mg/l IBA + 0.5 mg/l NAA in third weeks of culture.
(e) Acclimatized regenerated plants of one month old.
(f) Regenerated plants of four months old.

nodular callus was observed from nodal segments on MS basal medium supplemented with 1.5 mg/l BAP + 0.2 mg/l NAA within three weeks in *Scoparia dulcis*. The callus produced large number of shoots when sub cultured on MS medium with 0.5 mg/l BAP + 0.1 mg/l NAA. Direct regeneration using shoot tips and nodal explants was observed in different medicinal plants by other researchers (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).

Rooting in regenerated shoots of *Scoparia dulcis* was achieved at 85.2 percent when the excised shoots were cultured individually on root induction medium consisting of half-strength MS medium with 0.5 mg/l IBA + 0.5 mg/l NAA (Table II). Roots initiated by third weeks of culture. In this medium the highest percent (85.2) was responded and maximum number (13.4±0.24) of root were formed at the cut end of microshoots within four weeks of culture. Use of auxins singly or in combination for rooting was also reported by different authors (Sahoo and Chand 1998; Ajithkumar and Seeni 1998; Rai 2002, Baskaran and Jayabalan 2005; Sivakumar and Krishnamurthy 2000; Hassan and Roy 2005; Rahman *et al.* 2006; Baksha *et al.* 2007).

Table II. Effect of half strength MS medium with different concentrations of auxin(s) on root formation in regenerated shoots of *Scoparia dulcis.*

Growth regulators (mg/l)			Shoots producing roots (%) (±SE)	No. of roots/shoot (±SE)
IBA	NAA	IAA		
0.5			67.2±1.53	11.8±0.37
0.75			63.2±1.46	09.2±0.37
1.0			57.8±1.85	09.6±0.51
	0.5		71.0±0.10	09.2±0.37
	0.75		54.2±1.53	08.0±0.71
	1.0		59.4±1.08	08.2±0.37
0.5	0.5		85.2±0.86	13.4±0.24
1.0	1.0		82.0±0.71	11.8±0.37
0.5		0.5	65.2±1.16	09.8±0.58
1.0		1.0	61.4±0.75	08.6±0.51
0.5	0.5	0.5	62.6±0.93	09.2±0.73
1.0	1.0	1.0	54.4±1.63	09.6±0.51

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

After eight weeks the rooted shoots (Fig 1d) were transferred to pots. None of the plantlets survived when directly transferred from rooting medium to the pot under natural conditions. About 85 percent of the transplanted plants of *Scoparia dulcis* survived if the plantlets in the rooting medium were kept 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 ± 2^{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 1e, 1f).

After three weeks, plants were transferred to an open place and gradually acclimated to outdoor conditions, where 80 percent plants 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). Likewise it could be possible to propagate important medicinal plants for cultivation and sustainable use and consequently to conserve them from their extinction.

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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. 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 (1972) The Wealth of India: Raw Materials. CSIR, New Delhi, India.Vol. ix: Rh-So, pp 260-261.

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. Ind. Res.* **42**(1) : 37-44.

Escandon A. S., Miyajima I., Alderete M., Hagiwara J. C., Facciuto G., Mata D. and Soto S. M. (2005) Wild ornamental germplasm exploration and domestication based on biotechnological approaches. *In vitro* colchicines treatment to obtain a new cultivar of *Scoparia montevidiensis*. *Electron Journal of Biotechnology* **8**(2): 1-8.

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. C*hinese 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.

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.

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