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Callus Induction and High Frequency Regeneration of Plantlets of *Scoparia dulcis* L., a Perennial Medicinal Herb, Through Auxiliary Shoot Proliferation

A.K.M. Sayeed Hassan, Farhana Afroz, Laila Shamroze Bari, John Liton Munshi, Miskat Ara Akter Jahan and Rahima Khatun

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

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Abstract

Green compact nodular callus was observed within three weeks from nodal segments of a perennial medicinal herb *Scoparia dulcis* L. on MS basal medium supplemented with 1.5 mg/l BAP + 0.2 mg/I NAA. The callus produced large number of shoots when subcultured on MS with 0.5 mg/l BAP + 0.1 mg/l NAA. *In vitro* raised shoots rooted on half strength of MS with 1.0 mg/l IBA + 1.0 mg/l NAA. For acclimatization and transplantation, the plantlets in the rooting culture tubes were kept in normal room temperature for seven days before transplanting in pots where plantlets were reared for three weeks. The survival rate of plantlets was found to be 85%. Regenerated plants were morphologically uniform having normal leaf shape and growth.

Introduction

Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds (Chand et al. 1997). Among the world's 25 best selling pharmaceutical medicines, 12 are plant derived (O'Neill and Lewis 1993). *Scoparia dulcis* L. commonly known as 'Chinigura' belongs to 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 *Scoparia* species were noted from Argentina (Escandon et al. 2005). Among them *Scoparia dulcis* is abundandly distributed in many tropical countries like India and Bangladesh. Leaf of *Scoparia dulcis* is used as a cure for gastric ulcer and 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 cooling 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 sugar levels to drop below normal and reduction of both blood and urine sugar occur gradually. Amellin is reported to raise the lowered alkali in diabetics, and reduce iron content of serum and acetone bodies in blood.

In accordance with the World Health Organization, more than 20,000 plant species are used medicinally, while 80% of the world's total population rely chiefly on herbal traditional medicine as their primary health care; even in the developed countries like USA, plants are the source of ingredients in one-forth of the prescriptions delivered by pharmacists (Phillipson 1990). However, with the increasing use of medicinal plants in many countries, and with the accelerating destruction of natural resources in the tropics, it has become clear that the exploitation of medicinal plants must be accompanied by conservation measures. Otherwise these plants become depleted as resources or may even face extinction (Hamann 1991). Thus it is important to regenerate the affected plants artificially, which having beneficial effects on the ecosystems. Particularly when conventional propagation methods lead to unsatisfactory results, plant tissue culture techniques may be a valuable tool for the production of large amount of genetically identical plantlets for further field culture as conservation (Wawrosch and Kopp 1999).

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* to rapidly disseminate superior clones once they are identified. Tissue culture technique can play an important role in the clonal propagation of elite clones and

germplasm conservation of this medicinal herb. There have been a few reports to date on *in vitro* propagation of *Scoparia dulcis* using nodal explants derived callus tissue. However, in Bangladesh, there is no 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 *in vitro* propagation of this important medicinal herb through callus 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 *Scoparia dulcis* L. was collected from BCSIR campus, Dhaka and used as source of explants. Shoot tips and nodal segments 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 8 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 basal medium was used for shoot proliferation and adventitious shoot regeneration and half strength of MS was used for *in vitro* rooting. All media were supplemented with 30 g/l sucrose, 7 g/l agar (Difco) and dispensed into 15 × 150 mm culture tubes and 250 ml conical flasks. The pH of the media was adjusted to 5.8 before autoc1aving at 121°C for 20 min. The cultures were incubated for a 16 h photoperiod at 24 ± 2°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 BA and 0 - 2.5 mg/l Kn were supplemented into MS to select the their best concentration for shoot induction. In the second set, combination of BA - NAA (0 - 2.5 mg/l) and BA - IAA (0 - 2.5 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 onto half strength of MS 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°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

Within seven to 15 days of culture callus was formed at the cut surface of nodal explant on MS supplemented with 0.5 - 2.0 mg/l BAP either alone or in combination with 0.1- 0.5 mg/l NAA or IAA (Table 1). Maximum (82.2%) callus was observed on MS with 1.5 mg/l BAP + 0.5 mg/l NAA after two weeks (Fig. la). In this combination deep green compact and nodular callus developed after four weeks of culture (Fig. 1b). Initiation of shoot buds was observed from the same callus when subcultured on MS + 0.5 mg/l BAP + 0.1 mg/l NAA after six weeks (Fig. 1c). Proliferation of callus along with profuse shoot bud formation on the same medium was obtained after eight weeks (Fig. 1d). Shoot differentiation and profuse shoot formation were found to be best from callus on the same medium after ten and 12 weeks of culture (Fig. 1e, f). However, BAP with NAA was found to be more effective than BAP with IAA for callus induction (Table 1). According to Preece et al. (1991), callus forms frequently at the basal cut ends of nodal explants on cytokinin-enriched medium in species exhibiting strong apical dominance.

The highest number of shoots was 29.2 ± 5.44 per culture and length of shoot was 7.2 ± 0.61 developed on MS with 0.5 mg/l BAP + 0.1 mg/l NAA (Table 1, Fig. 1f). There were differences in regeneration frequencies, number and length of shoots per culture in different combinations. As stated by Martin (2002) the high morphogenic efficiency of nodal segments derived callus may be due to the presence of some internal components from the pre-existing axillary buds that are essential for induction of caulogenesis. Shoot buds developed and elongated from callus culture on the same medium. This continued in two subsequent subcultures made up of identical constituents at an interval of 15 days. Shoot regeneration via a callus phase was the simplest way to induce somaclonal variation and thus pave the way for improvement of the species (Thorpe et al. 1991). Such indirect organogenesis was reported in many medicinal plant species including *Asparagus cooperi* (Ghosh and Sen 1989), *Bixa orellana* (Sha et al. 2002), *Plumbago zeylanica* (Das and Rout 2002), *Holostema ada-kodien* (Martin 2002), *Ananas comosus* (Akbar et al. 2003), *Rotula aquatica* (Martin 2003), *Gloriosa superba* (Sivakumer et al. 2003), *Phellodendron amurense* (Azad et al. 2005) and *Abrus precatorius* (Biswas et a1. 2007).

Rooting in regenerated shoots (85.2%) of *Scoparia dulcis* was obtained when the excised shoots were cultured individually on root induction medium consisting of half strength of MS with 0.5 mg/l IBA + 0.5 mg/l NAA (Table 2).

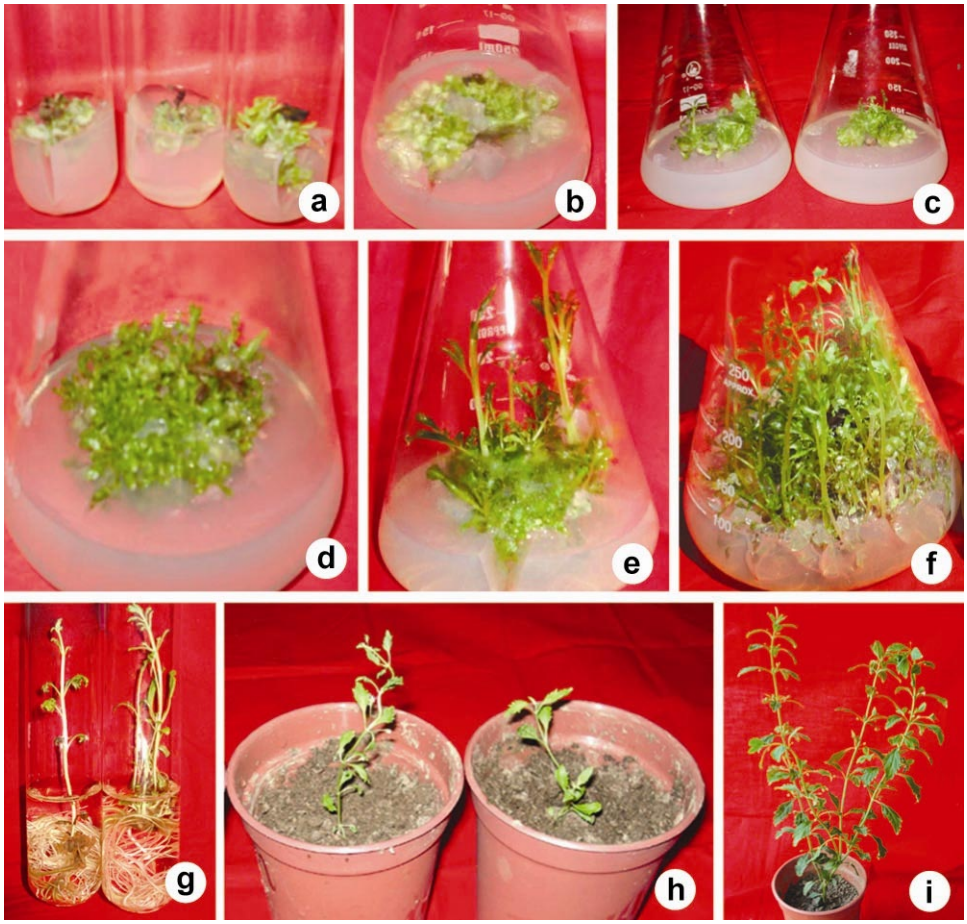

Fig. 1. *In vitro* plant regeneration from callus culture of *Scoparia dulcis.* (a) Callus formation started at the cut surface of nodal explant. (b) Deep green nodular callus expansion on the same medium after four weeks of culture. (c) Initiation of shoot buds from the same callus after six weeks of culture. (d) Proliferation of callus along with profuse shoot bud formation on the same medium after eight weeks of culture. (e) Shoot differentiation from callus on the same medium after ten weeks of culture. (f) Profuse shoot differentiation from callus on the same medium after 12 weeks of culture. (g) Rooting of *in vitro* regenerated shoots in half strength of MS in eight weeks. (h) Acclimatized regenerated plants of one month old. (i) Acclimatized regenerated plant of three months old.

Roots initiated by third weeks of culture. In this medium the highest per cent (85.2) of roots responded and their maximum number was 13.4 ± 0.24 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 1. Effect of different concentrations and combinations of growth regulators on MS for the adventitious shoot regeneration from the nodal callus of *Scoparia dulcis*.

Growth regulators (mg/l)			% of explants producing callus with shoots	Mean No. of shoots/culture (Mean ± SE)	Mean length of shoots (cm) (Mean ± SE)
BAP	NAA	IAA			
0.5			24.2	9.6 ± 0.51	2.6 ± 0.40
1.0			33.8	9.4 ± 0.51	2.2 ± 0.20
1.5			46.8	10.6 ± 0.40	2.6 ± 0.40
2.0			40.4	8.8 ± 0.37	2.2 ± 0.20
0.5	**0.1**		**81.2**	**29.2 ± 1.74**	**7.2 ± 0.58**
1.0	0.2		70.6	11.6 ± 0.51	2.8 ± 0.37
1.5	0.5		82.2	14.4 ± 1.29	3.2 ± 0.37
2.0	0.5		67.2	13.0 ± 0.71	2.6 ± 0.24
0.5		0.1	74.0	23.4 ± 0.75	6.2 ± 0.37
1.0		0.2	68.8	10.8 ± 0.37	3.4 ± 0.51
1.5		0.5	77.8	13.4 ± 0.75	3.6 ± 0.51
2.0		0.5	64.4	11.8 ± 0.37	2.8 ± 0.37

Data were recorded two months after inoculation. Results are mean ± SE of three experiments with 15 replications.

After eight weeks (Fig. 1 g) the rooted shoots were transferred to pots. None of the plantlets survived when directly transferred from rooting medium to the pot under natural conditions. About 85 per cent of the transplanted plants of *Scoparia dulcis* survived if the plants in the rooting culture tubes 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 per cent humidity. During this period of acclimation shoots elongated, leaves expanded and turned deep green and healthier (Fig. 1h).

After three weeks, plants were transferred to an open place and gradually acclimated to outdoor conditions, where 85 per cent plants survived. The technique described here appears to be a promising one of propagation of *Scoparia dulcis*. As the potentiality of shoot multiplication from callus continued for a long time, regenerates may be characterized by somaclonal variation. Several species of *Dubosia* (Lin and Griffm 1992), *Cuphea* (Millam et al. 1997), *Amaranthus* (Bennici et al. 1997), *Salvia* (Liu et al. 2000) and *Ananas comosus* (Akbar 2003) produced regenerates through callus-mediated adventitious shoot differentiation. Such regenerates may prove to be a potential source of

somaclonal variants, giving birth to traits of agronomic importance. The regenerated plants of *Scoparia dulcis* are currently being screened for agronomically useful genetic variants.

Table 2. Effect of half strength MS with different concentrations of auxin on root formation in regenerated shoots of *Scoparia dulcis*.

Growth regulators (mg/l)			% of shoots producing roots	Mean no. of roots/shoot (Mean ± SE)	Average length (cm) of roots (Mean ± SE)
IBA	NAA	IAA			
0.5			67.2	11.8 ± 0.37	2.3 ± 0.20
0.75			63.2	9.2 ± 0.37	2.6 ± 0.29
1.0			57.8	9.6 ± 0.51	2.3 ± 0.20
	0.5		71.0	9.2 ± 0.37	2.4 ± 0.19
	0.75		54.2	8.0 ± 0.71	2.3 ± 0.20
	1.0		59.4	8.2 ± 0.37	1.9 ± 0.10
0.5	**0.5**		**85.2**	**13.4 ± 0.24**	**3.5 ± 0.22**
1.0	1.0		82.0	11.8 ± 0.37	2.6 ± 0.19
0.5		0.5	65.2	9.8 ± 0.58	2.0 ± 0.16
1.0		1.0	61.4	8.6 ± 0.51	2.1 ± 0.24
0.5	0.5	0.5	62.6	9.2 ± 0.73	2.0 ± 0.16
1.0	1.0	1.0	54.4	9.6 ± 0.51	1.6 ± 0.19

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

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