ESTABLISHMENT OF AN EFFICIENT METHOD FOR MICROPROPAGATION OF AN IMPORTANT MEDICINAL HERB (*Scoparia dulcis* L.) FROM SHOOT TIPS AND NODAL SEGMENTS

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ABSTRACT

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The present experiment was conducted in the Laboratory of Biotechnology, Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, during January to August 2008 to establish a standard protocol for plant regeneration as well as acclimatization of plantlets. Shoot tips and nodal segments were cultured on MS medium supplemented with different concentrations of cytokinins for multiple shoot induction. The highest number of shoots (85.3) per explant was recorded on the MS medium supplemented with 1.0 mg/l BAP from nodal segment after 8 weeks of culture. The highest number of roots (21.5) per shoot was observed in the MS medium having 0.5 mg/l NAA. The well rooted plantlets were acclimatized and successfully transferred to natural condition, where 80% plantlets were survived.

Key words: Micropropagation, shoot tips, nodal segments,

INTRODUCTION

Scoparia dulcis is an important medicinal plant, belonging to the family Scrophulariaceae. It is locally known as Bondhane. The plant is a small, much branched, glabrous, leafy annual herb or under shrub with erects or ascending branches (Stone and Benjmin, 1970). The plant contains various kinds of biochemical compound such as, phenols, saponins, tannins, amino acids, flavonoids, terpenoids and catechnolamines (Ratnasooriya *et al.* 2005). Various parts of this plant has as an excellent medicinal uses, such as analgesis and anti-inflammatory activity (Ahmed *et al.* 2001), neurotrophic activity (Li *et al.* 2004), antiviral activity, antimalarial activity (Hayashi *et al.* 1990), anticancer activity (Nishino, 1993) and antidiabetic activity (Latha and Pari, 2005). In Brazil, various parts of the plant is used for abortions, bronchitis, cardiopulmonary disorders, coughs, diabetes, earache, excessive phlegm, eye problems, fever, gastric, disorders, hemorrhoids, hypertension, hyperglycemia, insect bites, jaundice, liver disorders, malaria, menstrual disorders, pain, upper respiratory disorders, skin problems, worms and wound (Cruz, 1995; Matos and Abreu, 1994; De Almeida, 1993; Coimbra and Raul, 1994). In Nicaragua it is used for anemia, childbirth, blood cleansing, burns, cough, diarrhea, fever, heart conditions, headache, infections, insect bites and stings, itching, liver disorders, malaria, menstrual disorders, snakebite, stomach disorders, venereal disease and for general tonic (Coee, 1996; Dennis, 1988).

Due to its non-judicial collection from natural resources without taking any care to its preservation and propagation, this particular plant is leading towards extinction. That is why; there is an urgent need to protect this plant. Simultaneously the *in vitro* culture methods are required for micropropagation and conservation of germplasm. It is conventionally propagated by vegetative cuttings but there is limited information of this plant regarding micropropagation through tissue culture. There are many reports on media compositions and protocol establishment for the *in vitro* propagation of several medicinal plans, such as *Adhatoda vasica* (Amin *et al.* 1997), *Rauvolfia serpentina* (Shyamol *et al.* 1994), *Polygonum hydropiper* (Hasan *et al.* 2006), *and Cassia alata* (Hasan *et al.* 2008). But there are no reports known to us on *in vitro* propagation of this valuable medicinal plant. In this experiment, a fruitful protocol was developed through multiple shoot induction from shoot tips and nodal segments. This protocol can be exploited for preservation of the valuable medicinal plant sources.

MATERIALS AND METHODS

Shoot tips and nodal segments of *Scoparia dulcis* L. were collected from Rajshahi University Campus, Bangladesh. The explants were washed first under running tap water for 20 minutes and treated with 1% Tween 80 for 8 minutes followed by repeated rinsing with sterile distilled water. Further sterilization was done under aseptic conditions in a Laminar Airflow Hood. Explants were surface sterilized with 0.1% (W/N) $HgCl_2$ for 5 minutes. Finally, the explants were washed thoroughly with autoclaved distilled water for several times to remove the traces of sterilant. The explants were cut into appropriate size and cultured on MS medium (Murashge and Skoog, 1962). Throughout the experiments full strength MS medium with 3% (W/V) sucrose and gelled with 0.8% (W/V) agar was used. The pH of all media was adjusted to 5.8 prior to autoclaving. The

cultures were incubated in a culture room at 25 ± 2°C with a photoperiod of 16 hour at 3000 lux light intensity provided by cool white fluorescent tubes.

The basal medium was supplemented with different concentrations of cytokinins. Rooting of shoots was achieved on MS medium supplemented with different concentrations of NAA or IBA. Well rooted plantlets were removed from the culture vessels, washed gently under running tap water and planted in plastic pots containing a potting mixture of sand, soil and humus in the ratio of 1:2:2. The potted plantlets were covered by polythene sheet to maintain suitable humidity. After sufficient acclimatization, the plantlets were transplanted into the pot containing soil, where 80% plantlets were survived.

RESULTS AND DISCUSSION

MS medium supplemented with different concentrations of cytokinin was used to find out the most suitable culture media for shoot multiplication from shoot tips and nodal segments of *Scoparia dulcis*. Multiple shoot initiation from both explants was observed within 8-13 days of inoculation (Figure A). From shoot tips the highest number of shoots per culture was 65.3 observed in the medium supplemented with 1.0 mg/l BAP followed by 42.2 in the medium consisting of 0.5 mg/l BAP. On the other hand, the lowest number of shoots per culture was 2.1 found in the medium supplemented with 4.0 mg/l KIN. From nodal segments the highest number of shoots per culture was 85.3 (Figure B) observed in the medium consisting of 1.0 mg/l BAP followed by 69.1 in the medium supplemented with 2.0 mg/l BAP. On the left hand, the lowest number of shoot per culture was 3.1 found in the medium having 0.5 mg/l KIN. The elongation of shoots was achieved in the same medium. The higher length of shoot was 6.8 cm in the medium having 2.0 mg/l KIN from nodal segment.

As per data recorded in Table 1, it can be concluded that MS medium supplemented with 1.0 mg/l BAP was suitable for multiple shoot initiation and 2.0 mg/l KIN was suitable for shoot elongation from nodal segment. Similar results were reported by many authors such as Karim *et al*. (2003) in *Chrysanthemum morifolium*, Akhter (1998) and Khan *et al*. (1994) in *Chrysanthemum*. On the other hand, Yadav *et al.* (1990) and Zaman *et al.* (1992) also reported the better effect of BAP (2.0 mg/l) in shoot multiplication and elongation in mulberry plant. Thus, nodal segments were better than shoot tips for shoot multiplication in the present study.

Regenerated shoots were inoculated for root induction. Different experiments were conducted with half and full strength of MS medium supplemented with different concentrations of auxins (NAA or IBA) singly. The highest number of roots per shoot was 21.5 observed in the half strength of MS medium having 0.5 mg/l NAA (Figure C) followed by 19.9 in the medium consisting of 1.0 mg/l IBA. On the other hand, the lowest number of roots per shoot was 9.8 observed in the full strength of MS medium supplemented with 0.1 mg/l NAA. The highest length of root was 4.6 cm found in MS medium consisting of 0.5 mg/l NAA followed by 4.5 cm in MS medium having 1.0 mg/l IBA. On the other hand, the lowest length of root was 2.9 cm observed in MS medium supplemented with 0.1 mg/l IBA.

In this experiment, it was found that NAA was more effective than IBA when used singly for rooting. Among the different concentrations tested in both half and full strength of MS medium supplemented with 0.5 mg/l NAA was found to be the best level for root induction and growth of roots (Figure C). Similar results were also observed by the Jha and Jha (1989) in *Caphaelis ipecacuanha,* Amin *et al.* (2003) in *Celtella asiatica*, Wakhlu and Barna (1988) in *Plantago ovata*.

After 35 days well rooted plantlets were obtained. The plantlets were removed from the culture vessels, washed gently under running tap water and planted in pots containing sterile sand, soil and humus in the ratio of 1:2:2 (Fig. D). After sufficient acclimatization, the plantlets were transplanted in the natural condition, where 80% plants were survived.

In the present investigation, a suitable protocol was established through multiple shoot induction from nodal segment and shoot tip explants of *Scoparia dulcis*. This protocol can be exploited for commercial propagation and conservation of other valuable medicinal plant resources.

Table 1. Effect of different concentrations of cytokinins (BAP, 2IP and KIN) singly in MS medium on shoot multiplication of *Scoparia dulcis* from shoot tips and nodal segments (data recorded after 8 weeks of culture and 20 explants were used for each treatment).

Treatments (mg/l)	Source of explants					
	Shoot tips			Nodal segments		
	Response of culture (%)	No. of shoots per culture	Length of shoots (cm)	Response of culture (%)	No. of shoots per culture	Length of shoots (cm)
BAP						
0.5	70	42.2±0.28	2.6±0.19	75	62.2±0.28	3.0±0.19
1.0	100	65.3±0.34	2.8±0.61	100	85.3±0.34	4.2±0.61
2.0	90	40.1±0.16	2.5±0.21	80	69.1±0.16	5.1±0.21
3.0	65	39.5±0.10	2.1±0.06	70	63.5±0.10	3.9±0.06
4.0	50	28.2±16	1.0±.06	55	36±0.16	2.0±0.16
2IP						
0.5	65	21.4±0.67	2.0±0.18	70	28.4±0.67	3.1±0.18
1.0	75	30.3±0.31	3.5±0.29	80	42.5±0.61	4.5±0.29
2.0	70	24.5±0.61	4.1±0.51	75	35.3±0.31	5.2±0.51
3.0	60	22.2±0.67	3.0±0.32	60	32.0±0.67	3.0±0.32
4.0	50	18±0.31	2.0±0.18	60	28.2±0.16	2.1±0.16
KIN						
0.5	40	2.4±0.45	3.1±0.39	40	3.1±0.45	3.9±0.39
1.0	50	3.2±0.04	4.1±0.47	55	4.5±0.04	5.2±0.47
2.0	65	6.8±0.73	3.8±0.16	70	7.8±0.73	6.8±0.16
3.0	45	4.2±0.38	4.4±0.82	50	5.2±0.38	5.4±0.82
4.0	35	2.1±0.91	4.0±0.19	45	3.4±0.91	2.9±0.19

Table 2. Effect of different concentrations of NAA and IBA in half strength and full strength of MS medium for root induction of *Scoparia dulcis* microcutting (data recorded after 35 days of culture).

Treatments (mg/l)	Media					
	Half strength			Full strength		
	Response of culture (%)	No. of roots per shoot	Length of root (cm)	Response of culture (%)	No. of roots per shoot	Length of root (cm)
NAA						
0.1	72	19.2±0.25	3.0±0.32	70	9.8±0.25	3.7±0.32
0.5	100	21.5±0.76	4.6±0.16	90	16.5±0.76	5.6±0.16
1.0	100	18.5±0.80	4.0±0.48	65	13.5±0.80	4.2±0.48
2.0	100	16.4±0.34	3.1±0.90	55	12.4±0.34	3.8±0.90
IBA						
0.1	65	15.4±0.09	2.9±0.41	45	9.8±0.09	2.9±0.41
0.5	100	17.6±0.47	4.0±0.17	62	12.6±0.47	3.9±0.17
1.0	100	19.9±0.77	4.5±0.78	75	13.9±0.77	4.5±0.78
2.0	100	14.3±0.53	3.8±0.35	60	10.5±0.53	3.7±0.35

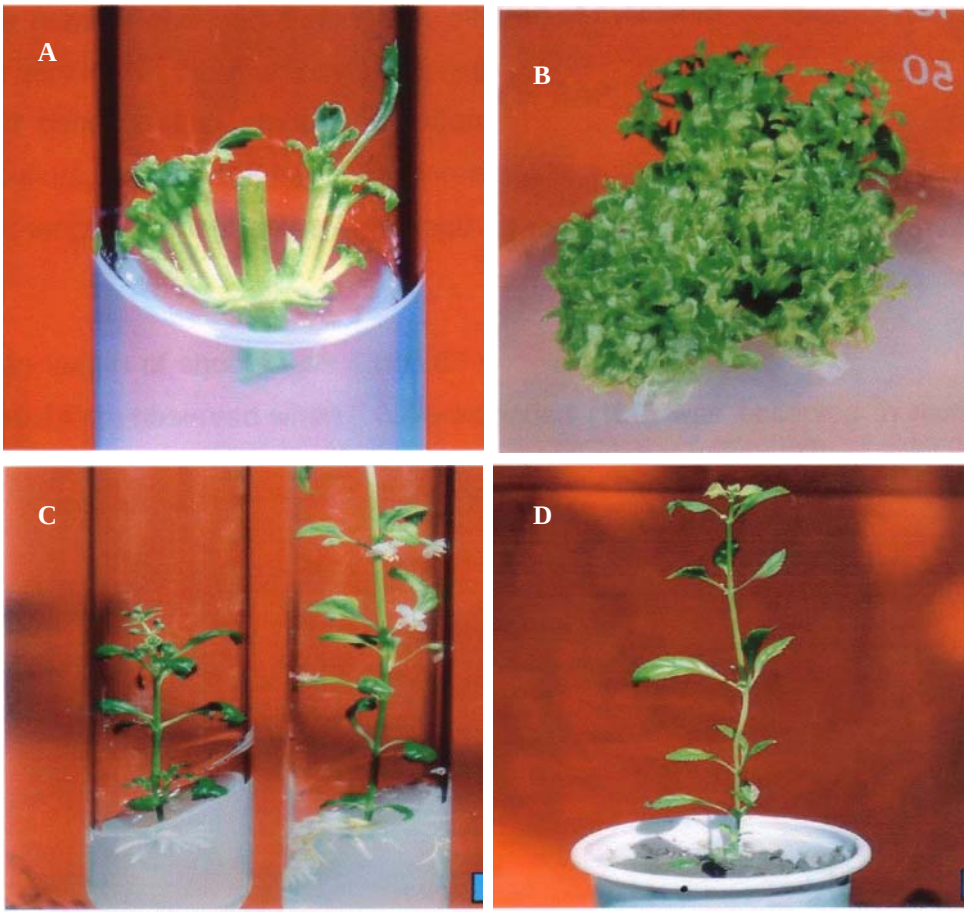

Figure 1. Plant regeneration through multiple shoot formation in *Scoparia dulcis*
A. Multiple shoots induction from nodal segment
B. Multiple shoots elongation
C. Root induction and elongation
D. Acclimatization

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