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Direct regeneration and *in vitro* flowering of *Scoparia dulcis* L.

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Abstract: This study reports a simple micropropagation protocol and thereby rapid multiplication of the useful medicinal plant- *Scoparia dulcis* L. Single node explants were inoculated on basal MS medium containing 3% (w/v) sucrose, supplemented with different concentrations and combinations of 6-benzylaminopurine (BAP), kinetin (KN), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and Naphthalene acetic acid (NAA) for direct plant regeneration. Maximum numbers of shoot (~22) were observed on the medium containing 0.5 mg/l BAP and 0.25 mg/l IAA after four weeks of culture. Regenerated shoots were separated and rooted on half strength MS medium supplemented with 0.5 mg/l of IBA alone for three weeks. Simultaneous regeneration of shoots and roots and *in vitro* flowering were achieved from the nodal explants on MS medium supplemented with 0.5 mg/l KN and 2.0 mg/l IAA. Well-developed complete plantlets were transferred on to specially made plastic cup containing soil rite. Acclimatized plantlets were successfully grown in garden soil.

Keywords: *Scoparia dulcis*, nodal explants, micropropagation, plant tissue culture.

Introductioon

S. dulcis is a perennial multi purpose medicinal herb distributed throughout tropical and subtropical regions which belongs to the family Scrophulariaceae (Riel *et al*., 2002, Latha *et al.,* 2004). Traditionally the fresh or dried plant has been used as a remedy for treating stomach ailments, hypertension, diabetes, inflammation, bronchitis, hemorrhoids, hepatosis, an analgesic and antipyretic agent (Riel *et al*., 2002, Ratnasooriya *et al*., 2005). Extracts of the plant contains antidiabetic activity (Pari *et al*., 2004), anticancer activity (Nishino *et al*., 1993), antimalarial activity (Riel *et al*., 2002), antiviral activity (Hayashi *et al*., 1988), neurotrophic activity (Li *el al.,* 2004) and anti-inflammatory activity (Ahmed *et al*., 2001). Conventional vegetative propagation on commercial scale has limitations (Thakur *et al.,* 1998). Therefore, micropropagation offers a reliable method for mass production of plants in a shorter time without seasonal constraints. So far, there is no report on *invitro* method of propagation for this plant in order to improve its cultivation. Therefore this first report on *in vitro* multiplication of *S. dulcis* through direct plant regeneration technique offers an effective alternative method of propagation for this important multipurpose medicinal plant.

Materials and methods

Explants were collected from moist deciduous forest, cut into nodal segments and used for induction of multiple shoots. The explants were washed with soap (soap powder) in running tap water for 1 hour. This is necessary to remove the exudates (phenolics, tannins and mucillages) present within the tissues. The explants were washed with Tween 20 (2%, w/v) and rinsed until traces of soap were removed. Later these explants were surface sterilized with 0.1% mercuric chloride (w/v) for 30 seconds and washed thrice using sterilized distilled water. Under aseptic conditions, explants were inoculated on basal MS (Murashige & Skoog, 1962) medium containing 3% (w/v) sucrose, supplemented with different concentrations and combinations of indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) or Naphthalene acetic acid (0.0, 0.25, 0.5 and 1.0 mg/l) either with 6- benzylaminopurine (BA: 0.0, 0.5, 1.0 and 2.0 mg/l) or kinetin (KN: 0.0, 0.5, 1.0 and 2.0 mg/l) for direct shoot regeneration and root induction. The pH was adjusted to 5.7 prior to the addition of 0.8% agar and autoclaved at 121°C (1.06 kg/cm2) for 15 min. Cultures were then incubated at 26±2°C with a 16-h photoperiod by cool white fluorescent tubes (Das et al., 1996) and 70-75% relative humidity (Mukherjee et al.,

Table 1: Shoot induction from node explants of S. dulcis at different concentrations of BAP, KN alone and in combination with IBA or IAA in MS medium

Growth regulators (mg/l)	% of explant showing response	No. of shoots	Average length of shoots (cm)
BAP			
0.5	60.0	5.4 ± 0.14	1.4 ± 0.23
1.0	68.0	7.2 ± 0.16	1.5 ± 0.26
1.5	82.0	12.3 ± 0.12	1.6 ± 0.17
2.0	95.0	14.6 ± 0.09	1.8 ± 0.21
KN			
0.5	64.0	4.6 ± 0.32	4.8 ± 0.46
1.0	79.0	5.3 ± 0.28	5.1 ± 0.25
1.5	91.0	8.1 ± 0.34	5.4 ± 0.28
2.0	97.0	8.8 ± 0.12	5.7 ± 0.18
BAP+NAA			
2.0+0.25	89.0	20.5 ± 0.18	1.7 ± 0.51
2.0+0.50	96.0	22.1 ± 0.26	2.0 ± 0.26
2.0+0.75	90.0	20.9 ± 0.15	1.7 ± 0.20
2.0+1.00	81.0	18.8 ± 0.13	1.2 ± 0.22
BAP+IBA			
2.0+0.25	95.0	16.6 ± 0.24	1.9 ± 0.24
2.0+0.50	95.0	16.2 ± 0.17	1.5 ± 0.32
2.0+0.75	88.0	15.7 ± 0.26	1.2 ± 0.28
2.0+1.00	85.0	13.4 ± 0.21	1.3 ± 0.09
KN+IBA			
2.0+0.25	92.0	1.3 ± 0.45	5.56 ± 0.52
2.0+0.50	94.0	1.4 ± 0.21	5.94 ± 0.28
2.0+0.75	90.0	1.2 ± 0.52	5.22 ± 0.14
2.0+1.00	83.0	1.2 ± 0.14	5.08 ± 0.16

20 explants and culture were maintained in each treatment and data (SE) were recorded up to four weeks of culture.

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Table 2: Root induction at different concentrations of IBA and IAA in MS medium from shoots of S. dulcis

Growth regulators (mg/l)	% of rooting response	No. of roots	Average length of roots (cm)
IAA			
0.25	95.0	3.8 ± 0.14	1.5 ± 0.14
0.50	100.0	4.2 ± 0.23	1.8 ± 0.24
1.00	97.0	4.0 ± 0.08	1.7 ± 0.18
2.00	98.0	3.7 ± 0.12	2.0 ± 0.14
IBA			
0.25	96.0	3.7 ± 0.20	1.9 ± 0.22
0.50	100.0	4.1 ± 0.26	2.1 ± 0.14
1.00	100.0	4.3 ± 0.12	1.8 ± 0.16
2.00	92.0	3.8 ± 0.16	1.7 ± 0.10

20 explants and culture were maintained in each treatment and data (SE) were recorded up to three weeks of culture

1991). For root induction, separated shoots were transferred to half strength MS basal medium supplemented with different concentrations of neither IBA nor IAA (0.0, 0.25, 0.50 and 1.0 mg/l) and 3% (w/v) sucrose. For hardening, the rooted plants were removed from the culture tubes, washed with sterile distilled water, and transferred to protrays with sterile cow dung: coco peat: sand (1:1:1 v/v/v). The plantlets were placed at 70% to 80 % humidity, 25 ± 2 °C under a 12-hours photoperiod for acclimatization. After the plants get acclimatized, the plants were transferred to pot with farmyard mixture: sand (1: 1 v/v) and placed in green house. After three weeks of development, these hardened plants were transferred to the field and the survival rate was recorded. Twenty cultures were used per treatment and each experiment was repeated at least three times. Percentage of success was scored four weeks after culture. Data collected were statistically analyzed and results presented in the tables.

Results and discussion

The nodal explants inoculated in MS medium individually supplemented with both BAP and KN showed remarkable response. In order to evaluate the synergistic effect different hormone combinations were tested in MS medium. Data on the effect of different concentrations of BAP, KN in combination with IBA and IAA in MS medium for multiple shoot induction of *S. dulcis* are presented in Table 1. The maximum number of shoot induction from the nodes of the explants was exhibited in a combination of BAP and IAA. Normally, other species like *S. montevidiensis* shows adequate response towards shoot regeneration in MS medium in the presence of BAP (Escandon *et al.,* 2005). The maximum average shoot length resulted when KN and IBA were used at a concentration of 2.0 and 0.5 mg/l respectively (Fig. 1).


Fig. 1


Fig. 2


Fig. 3

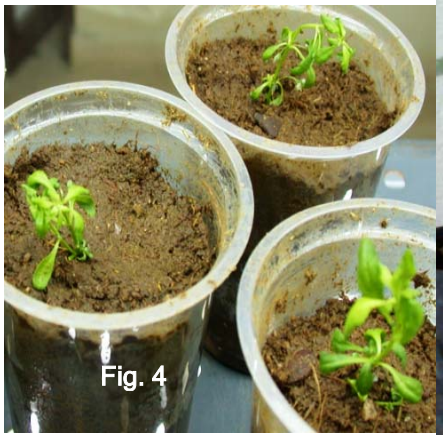
Fig. 4


Fig. 5

The mean values of root induction from shoots of *S. dulcis* cultured in MS medium with different concentrations of IBA and IAA are given in Table 2. In the case of IAA, maximum root induction was noticed at a concentration of 0.5mg/l (Fig. 2), whereas in MS medium with IBA showed maximum root induction at a concentration of 0.5mg/l. Regarding root length, there was not much difference between IAA and IBA treatments.

The mean values of simultaneous shoot and root induction of tissue cultured *S. dulcis* in MS medium with different concentrations of KN and IAA combinations are depicted in Table 3. The MS medium with 0.5 and 2.0 mg/l of KN and IAA respectively gave better result in respect of number of shoots, roots and flowers (Fig. 3).

During the process acclimatization and hardening, about 95% survival in chamber culture (Fig. 4) and about 100% survival in both greenhouse (Fig. 5) and field were noticed. The regenerated plants were phenotypically normal. Direct shoot multiplication is preferred for generating true-to-type plants than callus regeneration. This study reports a simple micropropagation protocol and the rapid multiplication of the useful medicinal plant- *S. dulcis* L by *in vitro* conditions. Shoots can be easily derived from node cultures on BAP containing medium and subsequently rooted on IBA containing medium. Both shoot and root can be derived from node cultures on KN and IAA

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Table 3: Effects of different concentrations of KN combination with IAA in MS medium for simultaneous shoot and root regeneration and in vitro flowering from node explants of S. dulcis

Growth regulators KN+IAA (mg/l)	explant response (%)	No. of shoots	Average shoot length (cm)	No. of roots	Average root length (cm)	No. of flowers
0.25+2.0	92.0	1.5 ± 0.34	5.45 ± 0.28	4.1 ± 0.22	1.81 ± 0.20	4.1 ± 0.18
0.50+2.0	97.0	1.8 ± 0.14	5.88 ± 0.10	4.4 ± 0.16	2.08 ± 0.10	4.8 ± 0.12
0.75+2.0	90.0	1.2 ± 0.28	4.64 ± 0.20	3.7 ± 0.26	1.47 ± 0.18	2.3 ± 0.24

20 explants and culture were maintained in each treatment and data (SE) were recorded up to four weeks of culture

containing medium. This approach offers a means for producing more identical plantlets from node explants of *S. dulcis* L.

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