In Vitro Cell.Dev.Biol.—Plant (2007) 43:436–441
DOI 10.1007/s11627-007-9090-1

Direct shoot regeneration from nodal explants of *Sida cordifolia* Linn

I. Sivanesan · Byoung Ryong Jeong

Received: 7 December 2006 / Accepted: 4 September 2007 / Published online: 9 October 2007 / Editor: Dudits Denes

Abstract A method was developed to initiate multiple shoots from mature nodal explants of *Sida cordifolia* Linn. High frequency of regeneration was achieved on Murashige and Skoog (MS) medium supplemented with 2.0 mg l^{-1} 6-benzylaminopurine, 0.5 mg l^{-1} α-naphthalene acidic acid, 1.0 mg l^{-1} adenine sulfate, and 10% (*v*/*v*) coconut milk. Multiple shoots were initiated within 21 d and the above media was capable of inducing the formation of more than 20 shoots from each explant. Regenerated shoots were successfully rooted on half-strength MS medium supplemented with 2.0 mg l^{-1} indole-3-butyric acid and 3% (*w*/*v*) sucrose. Rooted plantlets were established in soil. The regenerated plantlets showed no morphological differences from the parent material. This protocol could be useful for germplasm conservation, cultivation, and genetic improvement of *S. cordifolia*.

Keywords Adenine sulfate · Coconut milk · Nodal explants · Regeneration · *Sida cordifolia*

I. Sivanesan (✉)
Centre for Advanced Studies in Botany,
University of Madras, Guindy Campus,
Chennai 600 025, India
e-mail: isivanesan@yahoo.com

B. R. Jeong
Research Institute of Life Science,
Gyeongsang National University,
Jinju 660-701, South Korea

I. Sivanesan · B. R. Jeong
Department of Horticulture, Division of Applied Life Science,
Graduate School, Gyeongsang National University,
Jinju 660-701, South Korea

Introduction

Medicinal plants are of great interest as pharmaceutical industries depend in part on plants for the production of secondary compounds. *Sida cordifolia* Linn. is a herb belonging to the family Malvaceae. It grows to a height of 0.9–1.5 m and is extensively used as a common herbal drug (Kirtikar and Basu 1980; Anonymous 1988) in traditional medicine against chronic dysentery, asthma, and gonorrhea in the Indian subcontinent (Chopra et al. 1958; Yusuf and Kabir 1999). The plant is reported to contain ephedrine, vasicinol, vasicinone, and *N*-methyl tryptophan (Ghosh and Dutt 1930; Gunatilaka et al. 1980; Asha and Bannerjee 1985). Recently, cardiovascular effects (Medeiros et al. 2006), analgesic, antiinflammatory (Sutradhar et al. 2006), and hypoglycemic activities (Kanth and Diwan 1999) were reported from its leaves. Despite its importance, or perhaps because of it, *S. cordifolia* is disappearing in the wild as a result of continuous deforestation and extensive collection. *In vitro* conservation of traditional medicinal plant germplasm is important to support chemical analysis and pharmacological and genetic improvement programs. Plant tissue culture is an alternative method of propagation (George and Sherrington 1984) and is used widely for the commercial propagation of a large number of plant species, including many medicinal plants (Rout et al. 2000). However, only a few reports are available on plant regeneration of Malvaceae members (Hasson and Poljakoff-Mayber 1995; Zapata et al. 1999) and to date, there are no reports on plant regeneration from explants of *S. cordifolia*. This study describes a protocol for direct regeneration through nodal explants of *S. cordifolia*.

Table 1. Effect of different concentrations of cytokinins on shoot bud regeneration from nodal explants of *S. cordifolia*

Concentration (mg l^{-1})		Shoot induction (%)	Average number of shoots per explant±SD
BAP	KN		
0	0	0.0	0.0
0.5	0	54.6±0.4hi	2.2±0.7d
1.0	0	80.3±1.2bc	3.0±0.5bc
1.5	0	84.0±0.6ab	3.4±0.2cb
2.0	0	87.6±1.0a	5.6±0.8a
3.0	0	76.2±1.7d	4.8±1.1ab
5.0	0	70.0±0.8e	4.2±1.0ab
0	0.5	60.4±2.1g	2.0±0.7d
0	1.0	65.8±0.6f	3.2±1.0cb
0	1.5	69.2±1.7e	4.6±0.6ab
0	2.0	66.4±1.2ef	4.0±0.8ab
0	3.0	57.9±0.6h	3.8±0.7cb
0	5.0	56.5±2.0h	2.6±0.4cd

Nodal explants were cultured on MS medium supplemented with different concentrations of BAP and kinetin (KN). Data were recorded after 45 d. The experiments were conducted with a minimum of 25 replicates per treatment and were repeated 3 times. Data having the same *letter* in a *column* were not significantly different by Duncan's multiple comparison test ($P<0.05$).

Materials and Methods

Plants were collected from the Botany Field Research Laboratory, University of Madras, Chennai, India. Actively growing shoots were used as the explant source. The explants were surface sterilized with 70% (*v*/*v*) ethanol for 60 s and 0.1% (*w*/*v*) mercuric chloride for 8 min and then rinsed four times with sterile distilled water. Segments, each possessing one node, were prepared and inoculated on Murashige and Skoog (MS) (Murashige and Skoog 1962) basal medium supplemented with different concentrations of 6-benzylaminopurine (BAP) or kinetin (KN) (0.0, 0.5, 1.0, 1.5, 2.0, 3.0, and 5.0 mg l^{-1}) for shoot bud induction. For shoot multiplication, 2.0 mg l^{-1} BAP along with different concentrations of indole-3-acidic acid (IAA), indole-3-butyric acid (IBA), α-napthaleneacetic acid (NAA) (0.1, 0.5, 1.0, 1.5, 2.0, and 3.0 mg l^{-1}), coconut milk (CM), and adenine sulfate were tested. Individual regenerated shoots, 3–4 cm long, were excised from the shoot clump and transferred to half-strength MS medium containing various concentrations of IAA, IBA, and NAA for induction of rooting.

All media contained 3% (*w*/*v*) sucrose and were solidified with 0.8% (*w*/*v*) agar. The pH of the medium was adjusted to 5.8 using 1 N NaOH or 0.1 N HCl before autoclaving at 121°C for 15 min. The cultures were incubated at 25±2°C and 70–80% relative humidity under a 16-h photoperiod of 60 μmol m^{-2} s^{-1} photosynthetic photon flux density provided by cool white fluorescent light.

After 30 d, rooted plantlets were removed from culture, rinsed in sterile distilled water to remove media, and planted in 9×9 cm plastic pots containing sterilized soil, sand, and vermiculite (1:1:1, *v*/*v*/*v*). The potted plants were irrigated with 1/4 strength MS basal salt solution devoid of sucrose and myo-inositol every 4 d for a total of 4 wk. After this time, plants were shade-dried for 2 wk then transferred to the field.

Twenty-five cultures were used per treatment and each experiment was repeated three times. Data were analyzed by analysis of variance (ANOVA) to detect significant differences between means using the SAS computer package (SAS Institute, Cary, NC, Release 8.1). Means differing significantly were compared using Duncan's multiple range test at the 5% probability level. Variability around the mean was represented as ±standard deviation (SD).

Results and Discussion

As the mechanism of apical dominance has been demonstrated to be under the control of various growth regulators, the proportion of these regulators in the media can be ma-

Table 2. Effect of BAP in combination with different concentrations of auxins on shoot bud regeneration from nodal explants of *S. cordifolia*

Concentration (mg l^{-1})	Average shoot induction (%)			Average number of shoots per explant		
	IAA	IBA	NAA	IAA	IBA	NAA
0.1	88.0±1.0bc	89.8±1.2bc	90.6±0.6ab	6.2±1.1de	7.4±0.7cd	7.6±0.4cd
0.5	90.3±1.1ab	93.2±1.3ab	96.0±0.7a	6.8±1.2de	9.0±0.8ab	11.8±0.6a
1.0	92.0±0.8ab	84.6±1.6bc	91.0±0.4ab	8.2±0.6bc	6.8±1.1de	9.0±1.1ab
1.5	85.4±2.0bc	79.6±1.0cd	86.6±0.7bc	7.6±1.0cd	6.3±0.9e	7.4±1.1cd
2.0	80.6±0.7cd	75.2±1.7de	82.2±0.9bcd	6.2±1.1de	5.6±1.1f	7.2±2.0cd
3.0	73.2±1.1de	70.0±2.0e	78.0±1.0de	6.0.±0.8e	5.4±1.7f	6.0±0.4e

Nodal explants were cultured on MS medium supplemented with 2.0 mg l^{-1} BAP and the above growth regulators. Data were recorded after 45 d. Twenty-five replicates were used per treatment. Variability around the mean was represented as ±SD. Data having the same *letter* in a *column* were not significantly differed by Duncan's multiple comparison test ($P<0.05$).

nipulated to induce the regeneration of each meristem into viable shoots. In this study, growth regulator type, concentration, and the source of explants were assessed to determine optimal culture conditions to regenerate shoots of *S. cordifolia in vitro*. The response of nodal explants cultured on MS medium supplemented with various concentrations of BAP and KN is presented in Table 1. Nodal explants failed to develop shoot buds in growth regulator-free medium. In contrast, when the shoots of the same explant were grown on culture media containing cytokinin, axillary shoots developed precociously, which proliferated to form clusters of secondary and tertiary shoots. When MS medium was supplemented with different concentrations of BAP, multiple shoots emerged from the nodal explants starting 2 wk after culture. The highest average number of regenerated shoots obtained was 5.6 per explant on medium fortified with 2.0 mg l^{-1} BAP. Concentrations of BAP higher than 2.0 mg l^{-1} had a negative effect on the shoot bud regeneration (Table 1). The role of BAP in bud breaking has already been reported in many medicinal plants such as *Zingiber officinale* (Balachandran et al. 1990), *Piper* spp. (Bhat et al. 1995), and *Houttuynia cordata* (Handique and Bora 1999), and results from the present study confirm a similar response in *S. cordifolia* whereby BAP promotes formation of multiple shoots from nodal explants.

Nodal explants in this study started growing after 3 wk of culture on MS medium containing KN at 0.5–5.0 mg l^{-1}, and subsequently produced shoot buds. Explants cultured on 1.5 mg l^{-1} KN produced both the highest frequency (69%) and the greatest number of regenerated shoots per

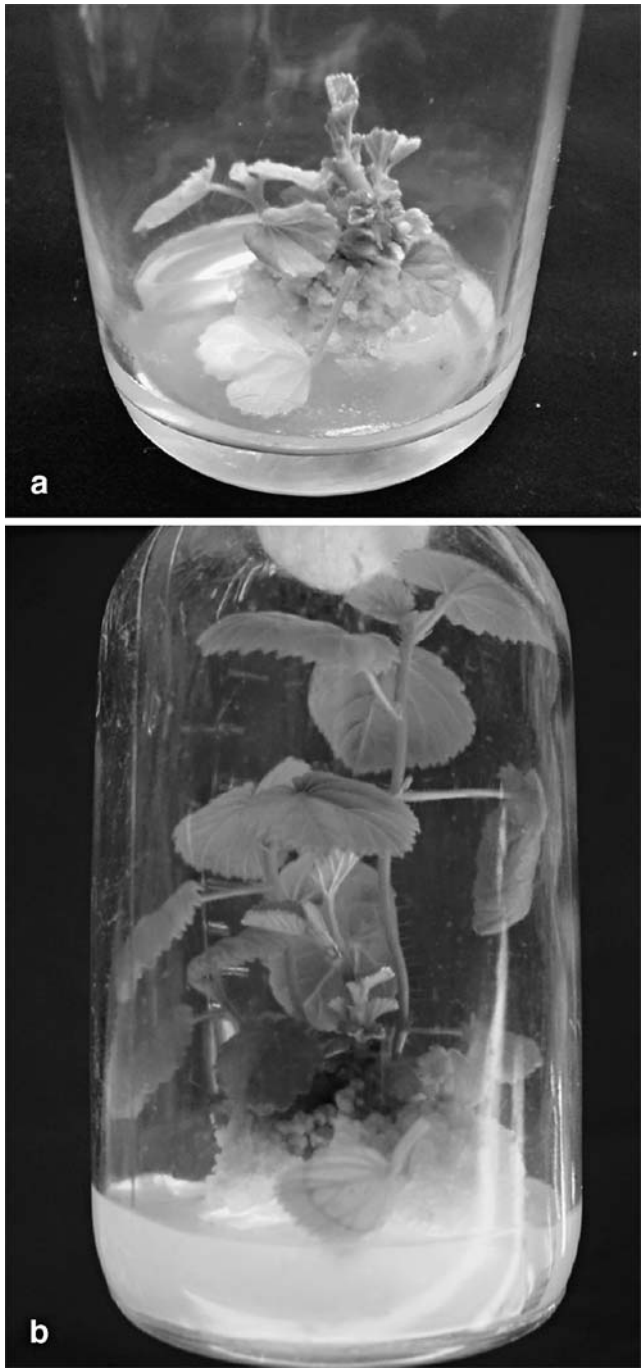

Figure 1. Shoot regeneration from nodal explants of *S. cordifolia*. *a* Shoot regeneration after 21 d culture on shoot induction medium. *b* Shoot regeneration after 45 d culture on shoot induction medium.

Table 3. Influence of coconut milk and adenine sulfate on shoot bud regeneration from nodal explants of *S. cordifolia*

Coconut milk (%, *v/v*)	Adenine sulfate (mg l^{-1})	Shoot induction (%)	Average number shoot per explant±SD
0	0.1	94.0±2.0c	12.2±0.7d
0	1.0	98.3±2.0ab	14.6±1.5c
5	0.0	100a	12.6±0.5d
10	0.0	100a	16.6±0.5b
10	1.0	100a	20.6±1.7a

Nodal explants were cultured on MS medium supplemented with 2.0 mg l^{-1} BAP, 0.5 mg l^{-1} NAA, and the above growth regulators. Data were recorded after 45 d. Twenty-five replicates were used per treatment. Variability around the mean was represented as ±SD. Data having the same *letter* in a *column* were not significantly differed by Duncan's multiple comparison test ($P<0.05$).

Table 4. Influence of node type on shoot bud regeneration from *S. cordifolia*

Node type	Shoot induction (%)	No. of shoots per explant±SD
Distal	82.0±4.8b	15.3±1.0b
Middle	100a	20.6±0.5a
Proximal	76.0±1.0c	14.1±1.3b

Nodal explants were cultured on MS medium supplemented with 2.0 mg l^{-1} BAP, 0.5 mg l^{-1} NAA, 1.0 mg l^{-1} adenine sulfate, and 10% (*v/v*) coconut milk. Data were recorded after 45 d. Twenty-five replicates were used per treatment. Variability around the mean was represented as ±SD. Data having the same *letter* in a *column* were not significantly differed by Duncan's multiple comparison test ($P<0.05$).

Table 5. Rooting response of shoots of *S. cordifolia* cultured on half-strength MS medium supplemented with auxins

	Average root induction (%)			Average number of roots per shoot±SD		
	IAA	IBA	NAA	IAA	IBA	NAA
0.5	59.0±1.0f	63.8±1.7e	42.9±1.1h	3.6±0.4ef	5.0±1.0cde	3.1±0.7f
1.0	68.4±1.7d	70.6±3.0cd	51.8±1.3g	4.0±1.1de	6.6±1.1bc	6.0±0.8bc
1.5	76.0±0.8c	89.6±1.1b	62.2±0.6e	4.4±0.7de	8.0±0.7ab	5.6±1.2cd
2.0	73.2±1.2cd	100a	60.6±0.4ef	5.6±0.6cd	9.2±0.7a	5.2±1.0cd

In vitro shoots (2–3 cm long) were cultured in half-strength MS medium with IAA, IBA, and NAA. Data were recorded after 35 d. Twenty-five replicates were used per treatment. Variability around the mean was represented as ±SD. Data having the same *letter* in a *column* were not significantly differed by Duncan's multiple comparison test ($P<0.05$).

explant (4.6). Although 54–87% of explants produced shoot buds in the presence of different BAP concentrations, only 56–69% of these explants exhibited a similar response to KN. Thus, whereas both cytokinins were capable of inducing shoot formation, BAP was found to be significantly more effective than KN in these studies (Table 1). Similar observations have been reported in other plants such as apple (Lundergan and Janick 1980), *Atrocarpus heterophyllum* (Rahaman and Blake 1988), *Dalbergia latifolia* (Raghav Swamy et al. 1992), *Chlorophytum borivilianum* (Purohit et al. 1994), and *Hibiscus cannabinus* (Herath et al. 2004).

To enhance shoot multiplication, different auxins were combined with the optimized BAP concentration of 2 mg l^{-1} described above. When an auxin was used in combination with BAP, numerous shoot buds were produced after 45 d of culture, in addition to callus at the cut end surface of the nodal explants. Both the frequency of shoot production and the average number of shoots produced per explant were significantly enhanced compared to the use of cytokinin only (Tables 1 and 2). All three auxins tested were capable of inducing more than 90% of the explants to respond positively in this manner, but the concentration of IAA required to achieve this was 1.0 mg l^{-1} compared to either 0.5 mg l^{-1} IBA or NAA. Inclusion of NAA at 0.5 mg l^{-1} in addition to BAP in the medium was capable of inducing an average of 11.8 shoots per explant and is the most effective growth regulator combination for shoot regeneration tested in this study (Table 2, Fig. 1). The use of NAA at relatively low concentrations to positively enhance shoot induction in the presence of cytokinin is in agreement with the results of Patnaik and Debata (1996) and Chen et al. (2001).

The beneficial effects of adenine sulfate and coconut milk in shoot induction was reported recently in *Plumbago zeylanica* (Wei et al. 2006). We also tested the effects of coconut milk and adenine sulfate on shoot bud proliferation in *S. cordifolia*. Incorporation of adenine sulfate at 1.0 mg l^{-1} in shoot multiplication medium did not improve shoot induction frequency but did favor shoot proliferation (Table 3). Increase in shoot number in the presence of adenine sulfate has been well documented (Rout et al. 1999; Das and Rout 2002) whereas coconut milk is also known to promote shoot bud multiplication in some culture systems (Grigoriadou et al. 2002; Mechanda et al. 2003). Among the different concentrations of CM tested in this study, the highest frequency of shoot bud induction and multiplication was observed in the presence of 10% (*v*/*v*) CM, which induced 100% of the nodal explants to produce multiple shoots, averaging of more than 16.5 shoots per explant. Combination of 10% CM and 1.0 mg l^{-1} adenine sulfate further enhanced the proliferation of shoot buds to reach more than 20 shoots produced per explant. Overall, this study therefore determined that MS medium supplemented with 2.0 mg l^{-1} BAP, 0.5 mg l^{-1} NAA, 1.0 mg l^{-1} adenine sulfate, and 10% (*v*/*v*) CM (Table 3) was highly

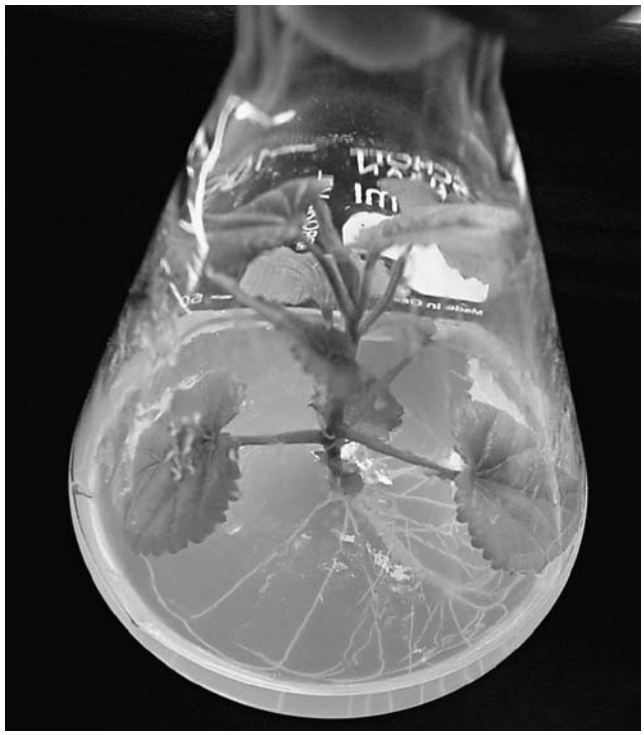

Figure 2. Rooted regenerated plantlet of *S. cordifolia*.

effective for the induction of shoot proliferation from nodal explants of *S. cordifolia*. On this media, 100% of explants were induced to produce an average of more than 20 shoots.

The determination of organogenesis also depends upon the source of plant tissue. Hence, in the present study, we used three types of nodes, types 1, 2, and 3, obtained from the distal, middle, and proximal ends of the explant, respectively. Among these nodal regions, 100% shoot induction was obtained with the middle portion whereas 82% of the distal and 76% of the proximal nodes produced shoots (Table 4). This may be because of the variation in the level of endogenous growth substances among the three nodal regions with the middle region having the optimum range when compared to the nodes at the distal and proximal regions.

Regenerated shoots of 2–3 cm long were excised and transferred to half-strength MS medium supplemented with different concentrations of auxins to investigate the effects of auxins on root induction (Table 5). Reduced strength of basal medium is often used for rooting of adventitious shoots (Hu and Wang 1983) and so was employed in this study. Roots started to emerge from the cut end of the shoots within 2 wk of transfer to rooting medium. The greatest response with 100% root induction and an average of 9.2 roots per shoot after 35 d culture was achieved on a ½ MS medium supplemented with 2.0 mg l^{-1} IBA (Fig. 2). Whereas 1.0 mg l^{-1} NAA also yielded a significant number of roots, IAA was found to be significantly less efficient for the induction of roots (Table 4). A high survival rate of plants, approaching 100%, was obtained by transferring rooted shoots to a soil, sand, and vermiculite (1:1:1, *v*/*v*/*v*) mix. A hardening period of 6 wk was then essential for the successful establishment of plants in soil condition. The regenerated plantlets did not show any morphological difference from those grown naturally with normal flowering and seed production.

Pharmaceutical companies largely depend upon the material procured from naturally occurring stands of medicinal plants, which are being depleted rapidly. The overexploitation of the natural population for medicinal use and lack of systematic efforts on the cultivation of this plant provides justification for the development of *in vitro* propagation method especially for this plant. This protocol could be used in the establishment of a large number of uniform plants of *S. cordifolia* through tissue culture for germplasm conservation, commercial cultivation, and also secondary metabolite production.

References

Anonymous. The Wealth of India. A Dictionary of Indian raw materials and industrial products, Newdelhi, Publication and Information Directorate CSIR. 323–324; 1988.

Asha B; Bannerjee NR. Polarographic studies on active constituents of *Sida cordifolia*. Cur. Sci. 54: 690–692; 1985.

Balachandran SM; Bhat SR; Chandal KPS. *In vitro* clonal multiplication of turmeric (*Curcuma* Spp.) and ginger (*Zingiber officinale* Rose). Plant Cell Rep. 8: 521–524; 1990.

Bhat SR; Chandal KPS; Malik SK. Plant regeneration from various explants of cultivated *Piper* species. Plant Cell Rep. 14: 398–402; 1995.

Chen CC; Chen SJ; Sagare AP; Tsay HS. Adventitious shoot regeneration from stem internode explants of *Adenophora triphylla* (Thunb.) A. DC. (*Campanulaceae*)—An important medicinal herb. Bot. Bull. Acad. Sin. 42: 1–7; 2001.

Chopra RN; Handa KL; Kapur LD. Chopra's indigenous drugs of India 2nd Edn., Academic Publishers. 409; 1958.

Das G; Rout GR. Direct plant regeneration from leaf explants of *Plumbago* species. Plant Cell Tiss. Organ Cult. 68: 311–314; 2002.

George EF; Sherrington PD. *In vitro*: Plant propagation by tissue culture, England, Eversley, Exegetics Ltd. 39–71; 1984.

Ghosh S; Dutt A. Chemical examination of *Sida cordifolia* Linn. J. Ind. Chem. Soc. 7: 825–820; 1930.

Grigoriadou K; Vasilakakis M; Eleftheriou EP. *In vitro* propagation of the Greek olive cultivar 'Chondrolia Chalkidikis'. Plant Cell Tiss. Organ Cult. 71: 47–54; 2002.

Gunatilaka AAL; Sotheeswaran S; Balasubramaniam S; Chandrasekara AI; Badrasriyani HT. Studies on medicinal plants of Sri Lanka. Planta Med. 39: 66–72; 1980.

Handique PJ; Bora P. *In vitro* regeneration of a medicinal plant—*Houttuynia cordata* Thunb. from nodal explants. Cur. Sci. 76: 1245–1247; 1999.

Hasson E; Poljakoff-Mayber A. Callus culture from hypocotyls of *Kosteletzkya virginica* (L.) seedlings. Plant Cell Tiss. Organ Cult. 43: 279–285; 1995.

Herath SP; Suzuki T; Hattori K. Multiple shoot regeneration from young shoots of kenaf (*Hibiscus cannabinus*). Plant Cell Tiss. Organ Cult. 77: 49–53; 2004.

Hu CY; Wang PJ. Meristem, shoot tip and bud cultures. In Evans DA, Sharp WR, Ammirato PV and Yamada Y (eds), Handbook of plant cell culture: Techniques for propagation and breeding vol. I. New York, Macmillan Publishing Co. 177–277; 1983.

Kanth VR; Diwan PV. Analgesic, antiinflammatory and hypoglycaemic activities of *Sida cordifolia*. Phytother Res. 13: 75–77; 1999.

Kirtikar KR; Basu BD. Indian Medicinal Plants. 2nd Edn., Bishen Singh Mahendra Pal Singh, Dehradun. 312; 1980.

Lundergan CA; Janick J. Regulation of apple shoot proliferation and growth *in vitro*. Hort. Res. 20: 19–24; 1980.

Mechanda SM; Baum BR; Johnson DA; Arnason JT. Direct shoot regeneration from leaf segments of mature plants of *Echinacea purpurea* (L.) Moench. *In Vitro* Cell. Dev. Biol.-Plant. 39: 505–509; 2003.

Medeiros IA; Santos MR; Nascimento NM; Duarte JC. Cardiovascular effects of *Sida cordifolia* leaves extracts in rats. Fitoterapia. 77: 19–27; 2006.

Murashige T; Skoog F. A revised for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 15: 473–497; 1962.

Patnaik J; Debata BK. Micropropagation of *Hemidesmus indicus* (L.) R. Br. through axillary bud culture. Plant Cell Rep. 15: 427–430; 1996.

Purohit SD; Dave A; Kukda G. Micropropagation of safed musli (*Chlorophytum borivilianum*), a rare Indian medicinal herb. Plant Cell Tiss. Organ Cult. 39: 93–96; 1994.

Raghav Swamy BV; Himabindu K; Lakshmi sita G. *In vitro* micropropagatipon of elite rosewood (*Dalbergia latifolia* Roxb.) Plant Cell Rep. 11: 126–131; 1992.

Rahaman MA; Blake J. Factors affecting *in vitro* proliferation and rooting of shoots of jackfruit (*Artocarpus heterophyllum* Lam). Plant Cell Tiss. Organ Cult. 13: 179–187; 1988.

Rout GR; Saxena C; Samantaray S; Das P. Rapid plant regeneration from callus cultures of *Plumbago zeylanica*. Plant Cell Tiss. Organ Cult. 56: 47–51; 1999.

Rout GR; Samantaray S; Das P. *In vitro* manipulation and propagation of medicinal plants. Biotechnol. Advances. 18: 91–120; 2000.

Sutradhar RK; Rahman AKMM; Ahmad M; Bachar SC; Saha A; Guha SK. Analgesic and Anti-inflammatory Principle from *Sida cordifolia* Linn. J. Biological Sci. 6: 160–163; 2006.

Wei X; Gou X; Yuan T; Russell SD. A highly efficient in vitro plant regeneration system and Agrobacterium-mediated transformation in *Plumbago zeylanica*. Plant Cell Rep. 25: 513–521; 2006.

Yusuf M; Kabir M. Medicinal plants of Bangladesh. Dhaka, Bangladesh, Council of Scientific and Industrial Research, CSIR. 226; 1999.

Zapata C; Srivatanakul M; Park SH; Lee MO; Salas GS; Smith RH. Improvements in shoot apex regeneration of two fiber crops: Cotton and kenaf. Plant Cell Tiss. Organ Cult. 56: 185–191; 1999.