Plant Tissue Cult. **14**(2) : 107-112, 2004 (December)

PTC

Rapid Proliferation of Multiple Shoots in *Solanum trilobatum* L.

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Key words : Solanum trilobatum, Rapid proliferation, Multiple shoots

Abstract

An efficient protocol was devised for rapid propagation of *Solanum trilobatum* L. from shoot tips and nodal explants. MS supplemented with different concentrations of BAP (2.22 - 13.32 µM/l) and Kn (2.32 - 13.82 µM/l) was tested for their efficiency in micropropagation. The highest rate of multiple shoot proliferation was observed on MS fortified with BAP (8.88 µM/l) followed by Kn (9.28 µM/l). The well-developed shoots were rooted on MS supplemented with IBA (9.48 µM/l).

Introduction

S. trilobatum L. is an important medicinal plant. The leaves contain rich amount of calcium, iron, phosphorus, carbohydrates, protein, fat, crude fibre and minerals (Anonymous 1972). This herbal plant is used as medicine for asthma, vomiting of blood and bilious matter, phlegmatic rheumatism, several kinds of leprosy. It is also antibacterial, antifungal, antimitotic and antitumourous (Subramanian and Madhavan 1983, Purushotaman et al. 1969, 1972 and 1987). Various members of this genus have been in focus for *in vitro* regeneration because of their high medicinal value owing to the presence of β-solanargine, solasodine and other closely related glyco-alkoloids (Guilietti et al. 1991). β-solanargine and solasodine are the major constituents in this species.

Only limited success has been reported for *in vitro* micropropagation and organogenesis of *S. trilobatum* (Arulmozhi and Ramanujam 1997). Pawar et al. (2002), reported cytokinins to be mainly responsible for shoot initiation in *S. surattense*. Plant regeneration through leaf-derived calli and organogenesis from stem explants has been achieved in *S. xanthocarpus* Schrad. & Wendl. (Baburaj and Thamizhchelvan 1991). Other *Solanum* species well studied for

in vitro morphogenetic response are *S. caciniatum* (Macek 1989) and *S. khasianum* (Bhalsing and Maheshwari 1997).

There are only a few reports on this plant for rapid multiplication, prompting the authors for attempting to propagate plants from shoot tips and nodal explants under *in vitro* conditions. This study describes the effects of BAP and Kn on shoot bud multiplication, elongation, rooting from shoot tip and nodal explants of *S. trilobatum*.

Materials and Methods

Shoot tips and nodal explants were collected from one-year-old healthy plants. Explants were washed with running tap water for 1 - 2 min and surface sterilized, initially in 70% ethanol followed by 0.1% (w/v) mercuric chloride for a few seconds and thoroughly rinsed three - four times in sterile distilled water. Explants were cultured on a MS with 30 g/l sucrose and 8.0 g/l Difcobacto agar (Hi-media Mumbay, India). For shoot regeneration different concentrations of BAP (2.22 - 13.32 µM/l) and Kn (2.32 - 12.82 µM/l) were tested. the medium pH was adjusted to 5.8 prior to autoclaving at 120_C for 15 min.

Cultures were maintained under cool-white fluorescent light (80 µ EM^{-2} S^{-1}) at 25 ± 2_C with 16 h photoperiod. The *in vitro* regenerated shoots were transferred to the rooting medium containing IBA (2.46 - 14.76 µM/l). Plantlets were transferred to plastic cups containing sterile red soil, sand and compost in the ratio of 1 : 1 : 1 in the greenhouse. At least 20 - 24 explants were cultured in each treatment and all the experiments were repeated three times.

Results and Discussion

Proliferation of multiple shoots was obtained with high frequency from shoot tips and nodes. These explants were capable of directly developing multiple shoots on MS containing differnt concentrations of cytokinins. Multiple shoot initiation from both explants was observed within 10 - 15 days after inocula-tion. The highest number of shoots (36/explant) was observed in the medium containing BAP (8.88 µM/l) followed by Kn (9.28 µM/l) with 29 shoots. Of the two cytokinins (BAP and Kn), BAP was found to be more suitable than Kn for initiation and proliferation of multiple shoot buds (Table 1).

The frequency of multple shoot bud induction was higher in nodes (36.33 ± 1.52) than shoot tips (28.00 ± 1.00) (Table 1; Figs. 1, 2). The elongation of shoots and proliferation of nodes were achieved on the same parental medium. In the present study, the relative effectiveness of BAP and Kn varied for *in vitro*

multiple shoot regeneration from shoot tips and nodes. BAP (8.88 µM/l) was found to be the best concentration for generation of maximum number of shoot buds (35 - 36). Shoots were harvested every 35 - 40 days and new shootlets were harvested periodically. Eighty one per cent of the plantlets produced roots on the rooting medium containing IBA (9.84 µM/l) after a week (Figs. 3, 4; Table 2). The *in vitro* regenerated plantlets were successfully transferred to plastic cups and then to the field (Fig. 5).

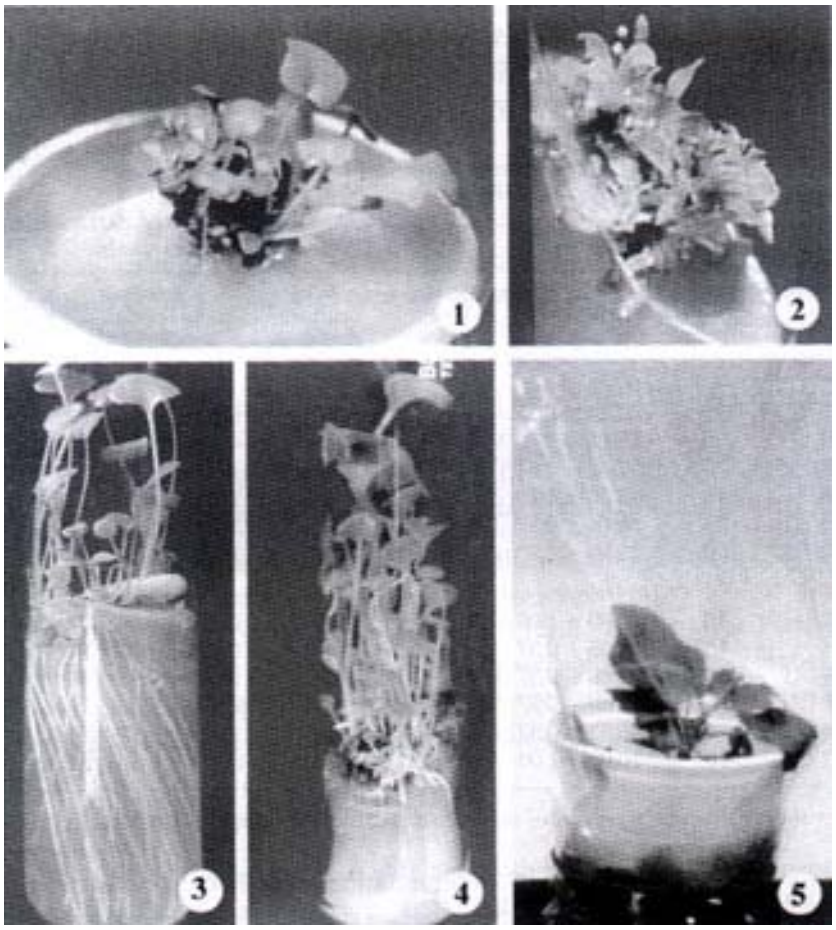
Table 1. Effect of different concentrations of BAP and Kn on multiple shoot induction from shoot tip and nodal explants of *S. trilobatum*.

Growth hormones (µM/l)	Shoot tip			Nodal explants		
	% of culture response	No. of multiple shoots/ shoot tip	Shoot length (cm)	% of culture response	No. of multiple shoots/nodal explant	Shoot length (cm)
BAP						
02.22	35	02.66 ± 0.57	1.66 ± 0.57	30	03.33 ± 0.57	02.66 ± 0.57
04.44	50	08.00 ± 1.00	4.33 ± 0.57	55	08.66 ± 0.57	05.66 ± 0.57
06.66	70	17.33 ± 1.52	7.00 ± 1.00	75	19.00 ± 1.00	08.66 ± 0.57
08.88	88	28.00 ± 1.00	9.66 ± 0.57	95	36.33 ± 1.52	11.00 ± 1.00
11.10	75	19.66 ± 0.57	7.33 ± 0.57	85	18.66 ± 1.52	06.66 ± 0.57
13.32	60	15.00 ± 1.00	5.00 ± 1.00	65	10.66 ± 1.52	04.66 ± 0.57
Kn						
02.32	30	02.00 ± 1.00	1.66 ± 0.57	35	03.00 ± 0.00	02.66 ± 0.57
04.64	45	06.33 ± 0.57	3.33 ± 0.57	50	08.00 ± 1.00	04.66 ± 0.57
06.96	65	14.66 ± 0.57	6.33 ± 1.15	70	16.00 ± 1.00	07.00 ± 1.00
09.28	85	25.00 ± 0.57	9.00 ± 1.00	90	29.00 ± 1.00	10.00 ± 1.00
11.60	70	16.33 ± 0.57	6.33 ± 0.57	75	17.33 ± 0.57	06.00 ± 1.00
13.82	60	10.00 ± 1.00	5.00 ± 1.00	50	08.00 ± 1.00	05.00 ± 1.00

Each value represents 20 replicates and each experiment was repeated at least thrice.

The capacity of shoot bud differentiation and shoot proliferation from shoot tip and nodal explants of *S. trilobatum* depended on hormonal variation. There was a good shoot bud initiation and proliferation response only in the presence of cytokinin and no response in the basal medium. Similar observation was made by Pattnaik and Chand (1996), in several medicinal plants. Sharon and Marie (2000) reported that the shoot tip and nodal explants were preferred over meristem to produce large number of genetically identical clones in *Bixa ovellana* L. BAP and Kn alone induced a higher frequency of multiple shoots. Similar results were obtained by Verma and Kant (1996), in *Emblica officinale* and Deka et al. 1999 in *Withania somnifera*. From our study it was clear that 8.88 µM/l BAP and 9.28 µM/l Kn were significantly more effective for inducing shoot organogenesis. Kulkarni and Rao (1999) reported that Kn did not support

the proliferation of multiple shoots in *Acorous calamus*. This result was in contrast to the present study where Kn was found to increase the frequency and the number of shoots.



Figs. 1 - 4. Multiple shoot induction and proliferation from: 1. shoot tip, 2. axillary node, 3. shoot elongation and rooting from shoots derived from shoot tips, 4. from axillary nodes and 5. harderned plantlets.

Pawar et al. 2002 reported that BAP and Kn individually and in combination induced a higher frequency of adventitious shoots from a single explant of *Solanum xanthocarpum*. This result was similar to that recorded in the present study. Well-developed shootlets when transferred to MS containing IBA induced roots. Similar effects of IBA were reported in *Ocimum americanum, O. canum* and *O. sanctum* (Pattnaik and Chand 1996) and also in *Heracleum candicans* (Wakhlu and Sharma 1999).

From our experimental data, it is evident that BAP and Kn are best suited for inducing multiple shoots and IBA for rooting. The nodal explants showed better response compared to shoot tips. In conclusion, this communication describes an efficient rapid propagation system of *S. trilobatum*.

Table 2. Effect of IBA on root induction in micropropagated plantlets of *S. trilobatum* L.

IBA (µM/l)	Shoot tip		Nodal explants	
	% of root induction from shoots	Average No. of roots/shoot	% of root induction from shoots	Average No. of roots/shoot
2.46	30.0	06.00 ± 1.00	36.06	06.66 ± 1.24
4.92	40.0	07.33 ± 0.57	52.0	10.00 ± 1.00
7.38	63.6	12.66 ± 0.57	72.0	13.66 ± 1.24
9.84	76.6	15.00 ± 1.00	81.3	16.00 ± 0.81
12.30	66.66	10.00 ± 1.00	78.6	11.66 ± 1.24
14.76	50.00	08.03 ± 1.15	60.0	08.33 ± 0.47

Each value represents an average of 20 replicates and each experiment was repeated atleast thrice.

References

Anonymous (1972) The wealth of India (Raw materials), CSIR, New Delhi **9**: 395.

Arulmozhi B and **Ramanujam MP** (1997) *In vitro* culture of *Solanum trilobatum* L. J. Swamy Bot. Club. **14**(1&2) : 55-56.

Baburaj and **Thamilzhchelvan P** (1991) Plant regeneration from leaf callus of *S. surattense*. Indian J. Exp. Biol. **29** : 391-392.

Bhalsing SR and **Maheswari VL** (1997) *In vitro* culture and regeneration of *Solanum khasianum* and extraction of Solasodine. J. Plant Biochem. Biotech. **6** : 39-40.

Deka AC and Kalita MC and **Baruah A** (1999) Micropropagation of a potent herbal medicinal plant, *Withania somnifera.* Environ. Ecology **17**(3) : 594-596.

Guilietti AM (1991) *Solanum elaeagnifolium* Cav. Silver leaf night shade *in vitro* culture and production of solasodine. *In:* Biotechnology in Agriculture Forestry, Medicinal and Aromatic Plants. YPS Bajaj (Ed.) Vol. **XIV** Springer-Verlag, Berlin, Germany. pp. 432-450.

Kulkarani VM and **Rao PS** (1999) *In vitro* propagation of sweet flag. J. Med. and Arom. Pl. Sci. **21**(2) : 325-350.

Pawar PK, Pawar CS, Narkhede BA, Teli NP, Bhalsing SR and **Maheswari VL** (2002) A technique for rapid micropropagation of *Solanum surattense* Burm. F. India J. Biotech. **1** : 201-204.

Pattnik SK and **Chand PK** (1996) *In vitro* propagation of the medicinal herbs. Plant Cell Reports **15** : 846-850.

Purushothaman KK, Saradambal S and Narayanaswamy V (1969) Chemical estimation of *Solanum trilobatum* L. Aust. J. Chem. **22**(7) : 1569-1570.

Sharon Marie (2000) *In vtiro* clonal propagation of ***Bixa orellana*** L. Cur. Sci. **78**(12) : 1532.

Subramanian SV and **Madhavan VR** (1983) Heritage of the Tamil siddha medicine. International Institute of Tamil Studies, Madras.

Verma B and **Kant U** (1996) Micropropagation of *Embilica officinale* Gaertz through mature nodal explant. J. Phytol. Res. **9**(2) : 107-109.

Wakhlu AK and **Sharma RK** (1999) Micropropagation of *Heracleum candicans* Wall. - a rare medicinal herb. *In vitro* cellular and Developmental Biology. Plant **35**(1) : 79-81.