Journal of Phytology 2011, 3(2): 18-23
Tissue Culture
ISSN: 2075-6240
Available Online: www.journal-phytology.com

REGULAR ARTICLE

MICROPROPAGATION OF A VALUABLE ETHNOMEDICINAL PLANT *STREBLUS ASPER* LOUR.

Kranthi Gadidasu, Pavan Umate, Mahender Aileni, Srinivasa Reddy Kota, Venugopal Rao Kokkirala, Kiranmayee Kasula and Sadanandam Abbagani*

Department of Biotechnology, Plant Biotechnology Research Unit,
Kakatiya University, Warangal – 506 009, India

SUMMARY

A micropropagation protocol is presented for conservation of critically threatened woody tree species, *Streblus asper* Lour. *In vitro* axillary bud proliferation followed by multiple shoot induction was obtained using mature nodal segments. Initially, explants were cultured on Murashige and Skoog's medium supplemented with different concentrations of BA (2.2, 4.4, 6.6, 8.9, 11.1 and 13.3 µM), Kn (2.3, 4.6, 6.9, 9.3, 11.6 and 13.9 µM) or TDZ (2.2, 4.5, 6.7, 8.90, 11.1 and 13.5 µM). These individual levels of cytokinins did not support *in vitro* shoot regeneration in *S. asper*. Combinations of cytokinins, Kn with BA or TDZ, significantly influenced shoot regeneration ability. The combination of Kn (4.60 µM) with BA (4.44 µM) evoked an optimum response towards shoot proliferation whereas, medium containing Kn (4.60 µM) plus TDZ (4.54 µM) induced multiple shoot formation. *In vitro* developed microshoots were rooted on MS half strength medium supplemented with 2.46 µM IBA. The plantlets established *in vitro* were transferred to pots containing sterilized soil and vermiculite (1:1) mixture and were hardened in the greenhouse with 70-75% survival rate.

Key words: Clonal propagation, Cytokinins, Mature nodal explant, Medicinal woody tree, Multiple shoot induction

Abbreviations: BA - 6-benzyladenine, IBA - indole 3-butyric acid, Kn - kinetin, µM - micro moles, MS – Murashige and Skoog TDZ - thidiazuron

Kranthi Gadidasu et al. Micropropagation of a Valuable Ethnomedicinal Plant Streblus asper Lour.. J Phytol 3/2 (2011) 18-23
*Corresponding Author, Email: nandamas@rediffmail.com, Tel: + 91-870-2571049, Fax: + 91-870-2438800

1. Introduction

Streblus asper Lour (Family Moraceae) is an important medicinal plant with various curative properties attributed to almost every part of the plant. It is a woody tree species distributed in tropical countries such as India, China, Sri Lanka, Malaysia, the Philippines and Thailand. This plant is known by various names like bar-inka, berrikka, rudi, sheora, koi, and siamese rough bush. It is also known as tooth brush plant as the branches are used for brushing teeth. The extracts isolated from different parts of this plant are used in traditional medicine for curing various disorders. For instance, the bark extract is been used as remedy for fever, dysentery, relief of toothache and antigingivitis (1). The root extract is applied to unhealthy ulcers, sinuses and used as an antidote to snake bite. The milky juice prepared from tender nodal and leaf cuttings has been used as antiseptic and astringent applied to chapped hands and sore heels (2). Anticancer, anti-microbial, anti-malarial and insecticidal activities were reported in this plant (3,4,5,6). The medicinal properties attributed to this plant are mainly due to the presence of several cardiac glycosides in different parts of this plant (7,8).

Currently, the plant is facing a threat of extinction due to destructive harvesting of plant parts for medicinal use as well as devastation of its natural habitat by deforestation. Besides, the conventional vegetative propagation methods for mass multiplication of this tree species are hampered due to low rates of seed

germination and poor rooting ability of the vegetative cuttings. Many rare and endangered plant species are propagated *in vitro* because they do not respond well to conventional methods of propagation. The media composition and qualitative and quantitative aspects of plant growth regulators play a vital role in micropropagation. Therefore optimization of these conditions is a prerequisite for *in vitro* related work. There are no reports available on *in vitro* propagation of *S. asper* that made us interested to develop micropropagation protocol for this threatened and medicinally important woody plant species. Micropropagated plants can thus act as source for germplasm conservation for this important tree species.

2. Material and Methods

The plant material was collected from the premises of the Kakatiya University campus, Warangal, India. Nodal segments were excised from tender parts of 10 year old plant. The explants were thoroughly washed under running tap water for 30 min then treated with 5% tween-20 for 5 minutes with constant stirring followed by 3-4 rinses in sterile distilled water. These explants were disinfected with 0.1% $HgCl_2$ for 5 minutes and rinsed 4-5 times with sterile distilled water. Explants were cultured on Murashige and Skoog's (9) medium containing 2% sucrose and 0.8% (w/v) agar (Himedia, India). The medium was supplemented with different concentrations of BA (2.2, 4,4, 6,6, 8.9, 11.1 and 13.3 µM), Kn (2.3, 4.6, 6.9, 9.3, 11.6 and 13.9 µM), or TDZ (2.2, 4.5, 6.7, 8.9, 11.1 and 13.5 µM) alone, or combinations of Kn with BA or TDZ were tested to induce shoot multiplication.

For root induction, elongated microshoots (3.5 cm, 4 week old) containing 3-4 leaves were transferred on to MS half strength medium supplemented with different concentrations of IBA (0.49, 1.48, 2.46, 4.90 and 9.80 µM). *In vitro* rooted shoots (3-4 week old) were weaned from the culture tubes and washed thoroughly in running tap water to remove agar and medium constituents. Individual shoots were potted in plastic jars containing sterilized soil and vermiculite (1:1) mixture. The potted plants were covered with polyethylene sheets to reduce the loss of moisture and hardened in the greenhouse (28°C day, 24°C night and 65% RH).

All media pH was adjusted to 5.8 with 0.1N NaOH before adding agar and was autoclaved at 121°C for 15 min. All cultures were incubated at 25±2°C with 16 h photoperiod under white fluorescent light (65 µE/m2/s). All data were statistically analysed using analysis of variance (ANOVA) and the means were compared using Duncan's multiple range test (DMRT). For *in vitro* shoot multiplication, 10 explants were used in each of two replicates for each treatment and the experiment was repeated twice. Rooting data was obtained from 10 explants per treatment in two replicates and the experiment was repeated twice

3. Results

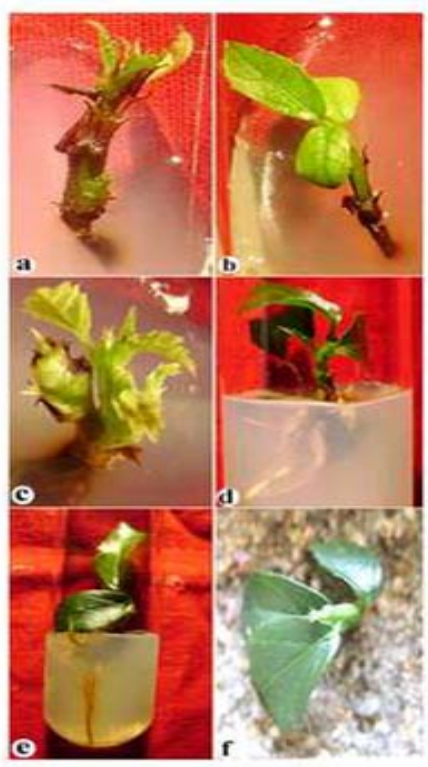
MS medium supplemented with different levels of BA, Kn or TDZ were tried to induce shoot proliferation from nodal explants of *S. asper.* Although nodal explants showed axillary bud sprouting on higher concentrations of BA (6.6, 8.9, 11.1 and 13.3 µM), Kn (6.9, 9.3, 11.6 and 13.9 µM), or lower levels of TDZ (2.2, 4.5, 6.7 and 8.9 µM) however these levels failed to induce shoot formation. Combined effect of cytokinins, Kn in combination with BA or TDZ, was tested on *in vitro* nodal proliferation and shoot development in *S. asper*. Interestingly, the above cytokinins when combined resulted in axillary bud proliferation as well as shoot formation. A combination of Kn (4.60 µM) plus BA (4.44 µM) showed maximum (90%) regeneration efficiency with increased mean length of shoot (3.5±0.09) (Table 1 and Fig. 1a and b). Other combinations of Kn with BA showed 65-75% of regeneration response and the average shoot length was in the range of 2.0 – 2.5 cm.

Table 1: Effects of different concentrations and combinations of BA, Kn and TDZ on shoot multiplication from nodal segments of *Streblus asper*

Growth regulators (µM)		% of cultures regenerated	Mean no. of shoots/explant	Mean length of shoot/explant
Kn	BA			
4.60	2.22	65	1.0±0.0a	2.2±0.12a
4.60	4.44	90	1.0 ± 0.0a	3.5±0.09b
4.60	6.62	75	1.0 ±0.0a	2.5±0.10c
4.60	8.90	68	1.0 ±0.0a	2.0±0.09a
KN	TDZ			
4.60	0.45	52	2.0±0.16b	2.1±0.13a
4.60	2.27	60	3.0±0.24c	2.4±0.15a
4.60	4.54	90	5.0±0.36d	2.8±0.21c
4.60	6.80	70	2.0±0.15b	1.5±0.14d

Values are mean of 40 explants
In each column, mean followed by same letter were not significantly different ($p<0.05$) according to Duncan's multiple range test

Fig.1: *In vitro* multiplication of *Streblus asper* (a) axillary bud breakage from nodal explants on BA and Kn (b) shoot proliferation from nodal segments on Kn (4.60 µM) plus BA (4.40 µM) (c) multiple shoot induction from nodal segments on Kn (4.60 µM) plus TDZ (4.45 µM) (d) root initiation on MS half strength medium supplemented with IBA (2.46 µM) (e) microshoot with well developed roots (f) plant in soil under greenhouse conditions



Multiple shoots were induced when nodal explants were cultured on medium supplemented with Kn in combination with TDZ. The mean number of shoots per explant varied among treatments of Kn plus TDZ (Table 1). A significantly higher numbers of multiple shoots per explant (5.0±0.36) were obtained on medium supplemented with Kn (4.60 µM) in combination with TDZ (4.54 µM) (Fig. 1c). Other combinations of Kn (4.60 µM) with TDZ (0.45, 2.27 and 5.80 µM) also resulted in multiple shoot formation from nodal explant cultures however their number remained low as compared to Kn (4.60 µM) plus TDZ (4.54 µM) combination. Under these conditions, the mean length of the shoot per explant was in the range of 1.5-2.8 cm that remained low as compared to combination of optimal levels of Kn (4.60 µM) plus BA (4.44 µM) (Table 1).

Well developed shoots (4-5 week old) with 3-4 leaves (Fig. 1b) were transferred to half strength MS media supplemented with various levels of IBA (0.49, 1.48, 2.46, 4.90, 9.80 µM). Root initiation occurred after 10 days of culture at all concentrations of IBA (Fig. 1d). Presence of IBA in the medium induced brown compact callus at the base of microshoots. Thus rooting occurred from the

base of the callus and not directly from the cut ends of microshoots. Maximum numbers of roots were developed at low levels of IBA (2.46 µM). At this IBA concentration, well developed roots were formed after 3 -4 week of culture such that rooted microshoots were ready for the transfer to the greenhouse (Fig. 1e). *In vitro* rooted microshoots with minimal callus were transferred to pots containing sterilized soil and vermiculite (1:1) mixture and hardened in the greenhouse (Fig. 1f). Plantlets established in pots exhibited 70-75% survival rate.

4. Discussion

The purpose of this study was to develop an *in vitro* propagation method from mature nodes of *S. asper*, a medicinally important plant. In the present work we have, for the first time, established a rapid and reproducible method for high-frequency axillary shoot proliferation from mature node segments of *S. asper*, followed by establishment of regenerated plants in soil. Axillary shoot proliferation from mature node was dependent on the interaction between plant growth regulator concentrations in the medium. The two-way interaction of cytokinins, Kn in combination with TDZ or BA significantly influenced shoot regeneration ability. The combination of Kn (4.60 µM) with BA (4.44 µM) evoked an optimum response showing increased mean length of shoot whereas, multiple shoots were induced from nodal explants cultured on medium containing Kn plus TDZ.

Although considered to be a most potent among the different phenyl urea derivatives for woody plant tissue culture (10), TDZ could not improve shoot bud differentiation when used alone in case of *S. asper*. Similar observations were previously reported with other species like *Feronia limonia* (11,12). In this study, BA (6.6, 8.9, 11.1 and 13.3 µM) or Kn (6.9, 9.3, 11.6 and 13.9 µM) even when used at high concentrations failed to induce shoot proliferation in *S. asper*. Hence a combination of cytokinins was used to improve the shoot multiplication rate, percentage of explants forming shoots as well as the shoot length. Earlier studies have compared the effectiveness of TDZ and amino purine cytokinins on shoot induction at several concentrations and using different explant types (10,13). For some species, the combination of TDZ plus an amino purine cytokinin (usually BA) is more effective at shoot induction than either TDZ or BA alone (14,15,16). In the present investigation, interaction of Kn with TDZ or BA was established that resulted in efficient shoot regeneration. The use of combination of cytokinins exerted a significant effect on number of shoots developed as well as on the shoot length in *S. asper*. The maximum shoot multiplication rate (5.0 shoots/explant) was observed when nodal explants were cultured on Kn plus TDZ medium. Further, the *in vitro* regenerated shoots attained maximum length when nodal segments were cultured on Kn plus BA medium. The decrease in shoot length on medium supplemented with Kn plus TDZ as compared to Kn plus BA might have resulted from inhibitory effect of TDZ on shoot elongation (10,13). However, nodal segments incubated on medium supplemented with Kn + TDZ or Kn + BA produced healthy shoots and overall shoot quality did not differ much when these media formulations were used. The synergistic action of a combination of two or more cytokinins resulting in to shoot induction from various explants has also been reported for *Gymnocladus dioicus* L. (17), *Eclipta alba* (18), *Feronia limonia* L. (19), *Albizia odoratissima* (20) and *Momordica tuberosa* Roxb.(21).

The role of cytokinins in shoot differentiation from nodal segments was reported in several woody species but only few reports were successful in inducing organogeneis from mature node explants (22). Explants from juvenile plants were also used for raising *in vitro* cultures from several woody trees (23,24,25). Here we report on shoot induction and multiplication from mature node explants of *S. asper*.

For successful micropropagation protocol, establishment of *in vitro* rooting from microshoots is crucial, and IBA is preferably used for the development of adventitious roots *in vitro* (26,22,25). During our study, all levels of IBA induced *in vitro* root formation. With increasing concentrations of IBA (4.90

and 9.80 μM), there was an increase in the amount of callus formed from the cut ends of the microshoot. Low level of IBA (2.46 μM) resulted in development of maximum number of healthy roots with little callusing.

In conclusion, *in vitro* growth and development from nodal explants of *S. asper* was highly influenced by the type of cytokinin combination used for propagation. The double interaction of Kn with BA or TDZ influenced shoot regeneration ability. The results presented also demonstrate that mature nodal explants of *S. asper* offer great potential as a source tissue for shoot induction. The procedure reported in this study may facilitate improvement, conservation, and mass propagation of this medicinally important tree species.

Acknowledgements

Authors thank Dr. V.S. Raju, Plant systematics laboratory, Kakatiya University, Warangal for the help with identification of plant material.

References

1. Gaitonde BB, Vaz AX, Patel JK. 1964. Chemical and pharmacological study of root bark of *Streblus asper*. Ind. J. Med. Sci 18: 191-199.
2. Mukherjee K, Roy LN. 1983. Chemical examination of *Streblus asper* leaves. Int. J. Crude Drug Res 21: 189-190.
3. Rastogi RP, Dhawan BN. 1990. Anticancer and antiviral activities in Indian medicinal plants: a review. Drug Dev Res 19: 1-12.
4. Das MK, Beuria MK. 1991. Anti-malarial property of an extract of the plant *Streblus asper* in murine malaria. Trans R Soc Trop Med Hyg 85: 40-41.
5. Wongkham S, Laupattarakasaem P, Pienthaweechai K, Areejitranusorn P, Wongkham C, Techanitiswad T. 2001. Antimicrobial activity of *Streblus asper* leaf extract. Phytother. Res 15: 119-121.
6. Hashim MS, Devi KS. 2003. Insecticidal action of the polyphenolic rich fractions from the stem bark of *Streblus asper* on Dysdercus cingulatus. Fitoterapia 74: 670-676.
7. Saxena VK, Chaturvedi SK. 1985. Cardiac glycosides from the roots of *Streblus asper*. J. Nat. Prod. 48: 981-985.
8. Duh CY, Pezzuto JM, Kinghorn AD, Farnsworth NR. 1985. Plant anticancer agents. XLI. Cardiac glycosides from *Streblus asper*. J .Nat. Prod 48: 981-985.
9. Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15: 473-497.
10. Huetteman CA, Preece JE. 1993. Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell Tiss Organ Cult 33: 105-119.
11. Hiregoudar LV, Murthy HN, Hema BP, Hahn EJ, Paek KY. 2003. Multiple shoot induction and plantlet regeneration of *Feronia limonia* (L.) Swingle. Sci. Hort 98: 357-364.
12. Tang H, Ren Z, Reustle G, Krczal G. 2002 .Plant regeneration from leaves of sweet and sour cherry cultivars. Sci. Hort 93: 235-244.
13. Lu C. 1993. The use of thidiazuron in tissue culture. *In vitro* Cell. Dev. Biol - Plant 29: 92-96.
14. Kerns HR, Meyer MM Jr. 1986. Tissue culture propagation of Acer x freemanii using thidiazuron to stimulate shoot tip proliferation. Hort. Science 21: 1209-1210.
15. Sudarsono A, Goldy RG. 1991.Growth regulator and axillary bud position effects on *in vitro* establishment of *Vitus rotundifolia*. Hort. Science 26: 304-307.
16. Sarwar M, Skirvin RM. 1997. Effect of thidiazuron and 6-benzylaminopurine on adventitious shoot regeneration from leaves of three strains of 'McIntosh' apple (Malus x domestica Borkh.) *in vitro*. Sci. Hort 68: 95-100.
17. Geneve RL. 2005. Comparative adventitious shoot induction in Kentucky coffee tree root and petiole explants treated with thidiazuron and benzylaminopurine. *In vitro* Cell. Dev. Biol - Plant 41: 489–493.
18. Baskaran P, Jayabalan N. 2005. An efficient micropropagation system for *Eclipta alba* - A valuable medicinal herb. *In vitro* Cell. Dev. Biol.- Plant 41: 532-539.
19. Vyas S, Joshi N, Tak K, Purohit S. 2005. *In vitro* adventitious shoot bud

differentiation and plantlet regeneration in *Feronia limonia* L. (Swingle). *In vitro* Cell. Dev. Biol - Plant 41: 296-302.

20. Rajeswari V, Paliwal K. 2006. *In vitro* propagation of *Albizia odoratissima* L.F. (Benth.) from cotyledonary node and leaf nodal explants. *In vitro* Cell. Dev. Biol - Plant 42: 399-404.
21. Aileni M, Kota SR, Kokkirala VR, Umate P, Abbagani S. 2008. Efficient *in vitro* regeneration and micropropagation of medicinal plant, *Momordica tuberosa* Roxb. J. Herbs, Spices and Medicinal Plants 14: 3.
22. Ramesh M, Pavan U, Shyam Prasad S, Rao AV, Sadanandam A. 2002. *In vitro* regeneration of plants from mature nodal segments of *Terminalia arjuna* Bedd. Sericologia 42: 75-80.
23. Gurumurthi K, Jagdees S. 1992. Micropropagation of *Azadirachta indica* Juss. from buds of coppice shoots. In: Reddy, K., ed. Vegetative propagation and biotechnologies for tree improvement. Dehradun: Natraj Publishers: 137-140.
24. Naomita V, Dalal V, Ravishankar Rai. 2004. *In vitro* propagation of *Oroxylum indicum* Vent. a medicinally important forest tree J. For. Res 9: 61-65.
25. Ramesh M, Umate P, Venugopal Rao K, Sadanandam, A. 2005. Micropropagation of *Terminalia bellirica* Roxb. – A sericulture and medicinal plant. *In vitro* Cell Dev. Biol - Plant 41: 320-323.
26. De Klerk GJ, Ter Brugge J, Marinova S. 1997. Effectiveness of indole acetic acid, indole butyric acid and naphthalene acetic acid during adventitious root formation *in vitro* in Malus 'Jork 9'. Plant Cell Tiss. Organ Cult 49: 39-44.