J. Plant Biol. 40 (3) : 202~205 (1997)

Micropropagation of *Tribulus terrestris* L., an Important Medicinal Plant

Ali, G., M.H. Mughal, P.S. Srivastava and M. Iqbal*

Department of Botany, Hamdard University, Hamdard Nagar, New Delhi-110 062, India

Direct regeneration of shoots and roots through juvenile explants has been achieved in *Tribulus terrestris*. Cotyledonary leaves along with epicotyl segment from young seedlings were cultured on MS medium containing various concentrations of auxin with cytokinin and glutamine. A combination of 0.2 mgL^{-1} NAA, 0.5 mgL^{-1} BAP and 50 mgL^{-1} glutamine induced high frequency of shoot and root differentiation in 10 weeks. The callus also could be induced on the above medium from the cut end of radical segments. Morphogenic response such as per cent shoot and root differentiation was recorded at regular intervals.

Keywords: *Tribulus terrestris*, medicinal plants, micropropagation, direct regeneration

Tribulus terrestris L., commonly known as Caltrops or Devil's thorn, is a small ascending and prostrate herb of the family Zygophyllaceae occurring up to 3000 m altitude. Plants and dried spiny fruits are used in decoctions or infusion in cases of spermatorrhoea, phosphaturia, and diseases of genito-urinary systems such as dysuria, gonorrhoea, gleet, chronic cystitis, calculous affections, urinary and renal disorders and gravel (Anonymous, 1948). It is also useful in cough, heart disease, and suppuression of urine. The drug has diuretic properties (Kapoor, 1990), and shows antiurolithiatic activity (Anand *et al.*, 1994). Owing to its various potential applications in the indigenous systems of medicine, the apparent overexploitation of this species may cause a serious threat to its existence. Additionally, its seed germination is only 25%. Inevitably therefore, rapid multiplication of this important drug-yielding plant has become imperative. *In vitro* culture methods have proved successful for quick propagation of a number of species including the herbaceous medicinal plants such as *Datura innoxia* (Srivastava *et al.*, 1993), *Ammi majus* (Purohit *et al.*, 1995), *Artemisia annua* (Gulati *et al.*, 1996) and *Bacopa monniera* (Ali *et al.*, 1996). The present report addresses the micropropagation of *T. terrestris*. The suggested technique must facilitate rapid multiplication of this drug-yielding plant.

*Corresponding author: Fax +91-11-698-8874

MATERIALS AND METHODS

Fruits of *T. terrestirs* L. were collected from the plants growing in the Herbal Garden at Jamia Hamdard, New Delhi. Seeds were allowed to germinate under *in vivo* as well as *in vitro* condition.

In Vitro Seed Germination

Seeds were sterilized with 0.3% cetrimide (ICI) for 7 min, and with bavistin (1%) and tetracycline (0.1%) for 7 min, and then treated with 5% fresh solution of sodium hypochlorite for 5 min, and 0.1% mercuric chloride for 3 min, followed by a final rinse with 70% alcohol for 1-2 min. The sterilized seeds were thoroughly rinsed with sterile distilled water before inoculation. Murashige and Skoog's (1962) (MS) basal medium gelled with 0.62% agar (Qualigens India), and pH adjusted to 5.7 before autoclaving, was used throughout. The cultures were maintained at $25\pm2^{\circ}C$ and $55\pm5\%$ relative humidity under 16 h photoperiod provided by fluorescent and incandescent light (1500 lux). Per cent germination was monitored on alternate days for 10 days.

Direct Regeneration

Epicotyl and hypocotyl segments from fully grown seedlings were cut into 10 mm segments and

cultured on MS medium (sucrose 2%) containing various concentrations of NAA (0.1-0.2 mg.L^{-1}) with BAP (0.5-1.0 mg.L^{-1}) and glutamine (50 mg.L^{-1}). Per cent shoot and root differentiation was recorded fortnightly till 10 weeks.

RESULTS

In Vitro Seed Germination

Emergence of radicle was observed in 42% seeds after 2 days of inoculation on 1/2 MS medium. However, in only 6-7% seeds did the radicles grow further by the 5th day. The *in vitro* seed germination recorded at the end of the 10th day was 78%, compared to 25% *in vivo* (Fig. 1).

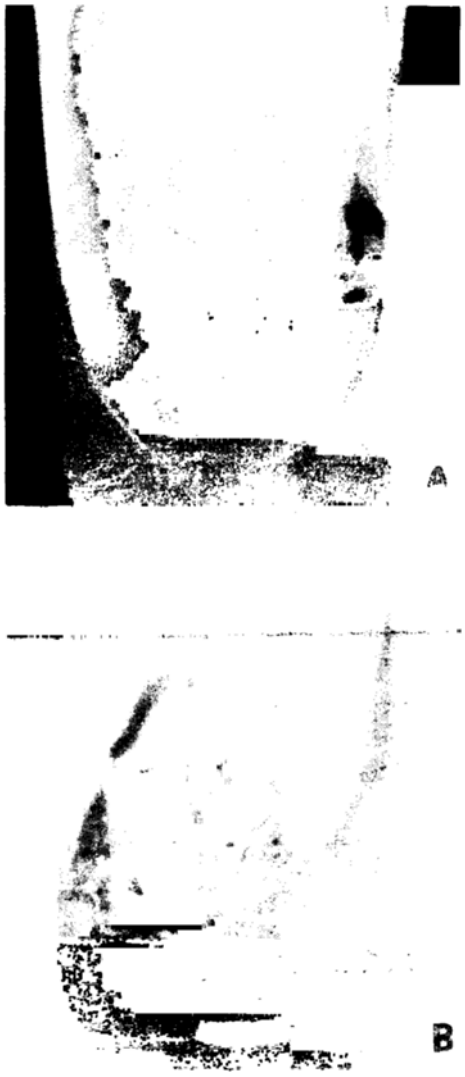

Fig. 1. *Tribulus terrestris*: (A) 10 day-old seedling on 1/2 MS basal medium; (B) 10-wk-old regenerants on MS medium supplemented with NAA (0.2 mg.L^{-1}), BAP (0.5 mg.L^{-1}) and glutamine (50 mg.L^{-1}).

Direct Regeneration

Direct regeneration of shoots and roots occurred when cotyledonary leaves along with epicotyl were cut into pieces and implanted on MS medium containing NAA, BAP and glutamine (Fig. 1B).

On MS medium supplemented with 0.1 mg.L^{-1} NAA, 0.5 mg.L^{-1} BAP and 50 mg.L^{-1} glutamine, shoot buds appeared in 10% cultures after 2 weeks. After 10 weeks, shoot regeneration was observed in 65% culture. However, on increasing the level of cytokinin (BAP) to 1.0 mg.L^{-1}, the shoot differentiation recorded in 15% cultures after 2 weeks rose to 72% after 10 weeks. Further, when the amount of NAA was raised to 0.2 mg.L^{-1} with BAP 0.5 mg.L^{-1}, the response was more promising, thus establishing this medium as the best for shoot regeneration. Here, 100 % cultures showed shoot regeneration after 10 weeks. However, on NAA (0.2 mg.L^{-1}) with BAP (1.0 mg.L^{-1}), shoots regenerated only in 64% cultures after 10 weeks (Table 1).

Callusing was obtained at the cut ends of radicle segments on MS medium with NAA (0.2 mg.L^{-1}), BAP (0.5 mg.L^{-1}) and glutamine (50 mg.L^{-1}) after 4 weeks. The callus was green and compact. When transferred to various media, it failed to regenerate.

Root Formation

Rooting also appeared on the above medium, showing a wide variation. On MS medium+NAA (0.1 mg.L^{-1})+BAP (0.5 mg.L^{-1})+glutamine (50 mg.L^{-1}), rooting was observed in 5% cultures after 2 weeks, and in 43% cultures after 10 weeks. Increased concentration of BAP (1.0 mg.L^{-1}) caused root formation in 5% cultures after 2 weeks and in 31% cultures after 10 weeks. Enhanced level of NAA (0.2 mg.L^{-1}) caused root formation in 29% cultures after 2 weeks and in 78% cultures by the end of the 10th week. Nonetheless, on BAP (1.0) and NAA (0.2), the per cent root formation was reduced (39% after 10 weeks) (Table 1). Glutamine was used as a nitrogen source. Thus regeneration of complete plantlets was achieved. The plantlets could be transferred to pots after sequential hardening.

DISCUSSION

Micropropagation has now emerged as the best approach regarding preservation of medicinal herbs. Re-

Table 1. Morphogenic response (formation of shoots and roots) of juvenile explants from *T. terristris* seedlings on MS (in mg.L^{-1})+NAA+BAP+glutamine (50 mg.L^{-1})

NAA	BAP	% Response									
		After 2 weeks		After 4 weeks		After 6 weeks		After 8 weeks		After 10 weeks	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0.1	0.5	10	5	22	17	30	26	45	32	65	43
0.1	1.0	15	5	27	15	32	21	49	26	72	31
0.2	0.5	29	23	39	38	60	50	80	60	100	78
0.2	1.0	11	4	24	15	32	24	46	30	64	39

generation of plants from cultured cells may occur via shoot-root formation (Srivastava *et al.*, 1993; Mohan *et al.*, 1995; Purohit *et al.*, 1995; Gulati *et al.*, 1996; Ali *et al.*, 1996) or somatic embryogenesis (Murashige, 1984) which is often the fastest method for cloning plant species. Advantage of *in vitro* micropropagation lies in its ability to produce in short time and limited space a much larger number of true-to-type individuals than is possible through conventional methods (Lauzer *et al.*, 1992). Several explants have been tried; the simplest and safest method of *in vitro* cloning of plants is the single node culture which produces numerous shoots capable of regenerating roots. Striking responses of *in vitro* differentiation in excised tissues depend on composition of the medium (Huang and Murashige, 1976) and the basic regulatory mechanism involves a balance between auxin and cytokinin (Skoog and Miller, 1957). Morphogenic responses exhibited in the form of shoots and/or roots are, therefore, correlative to a specific auxin/cytokinin ratio. One of the most influencing factors in adventitious organ (root/shoot) formation is the modulation of endogenous auxin to cytokinin balance (Thorpe, 1980). This is true for culture of explants from different families (Gunay and Rao, 1978; Sim *et al.*, 1990; Samantaray *et al.*, 1995). Implicitly, the initiation of a proliferating culture from the explant involves profound changes in the developmental state of the tissues and results in alteration in the basic architecture of cells/tissues leading to activation of even quiescent or fully-differentiated cell. The present study has shown that changes in the levels of NAA and BAP can significantly affect morphogenic events in *T. terrestris*. These results are corroborated by earlier findings of Jaiswal *et al.* (1989), Ali *et al.* (1996) and Quraishi *et al.* (1996). Rooting and transplantation of the plantlets to the field is the most important but difficult task in micropropagation (Murashige, 1974). Earlier studies recommended that rooting was achieved when the shoots which regenerated on various levels of NAA/BAP were transferred on various concentrations of IBA. However, in case of *T. terrestris* the rooting could be obtained on all combinations of NAA and BAP tried. Glutamine was used as a nitrogen source. Removal of glutamine from the MS medium resulted in yellowish cultures of *Bacopa monniera,* while its addition to the medium made the regenerants greenish (Ali *et al.*, 1996). Similar observation have been made for *T. terrestris* also.

Under *in vitro* conditions, shoot bud regeneration can be achieved either through callus or directly from the cultured explants by skipping over the callus phase. Because of the ease with which both kinds of regeneration can be obtained, the *T. terrestris* plant becomes a suitable material for a variety of studies. Direct regeneration of plantlets through juvenile explants in *T. terrestris* is a two-step process; shoot formation followed by rooting. Shoots differentiated in 100% cultures grown on MS medium with NAA (0.2 mg.L^{-1}), BAP (0.5 mg.L^{-1}) and glutamine (50 mg.L^{-1}). The subsequent root formation featured in 79% cultures. The results indicate that the requirement of auxin (*eg.* NAA) and cytokinin (*eg.* BAP) is important in determining the response in *T. terrestris*. In the present investigation it has been possible to regenerate plants from cotyledonary explants that could be transferred to soil.

Earlier reports (Erhun and Sofowora, 1986) have mentioned callusing from both, leaf and mature stem segments on MS medium with 0.55 mg.L^{-1} 2,4-D and 0.43 mg.L^{-1} kinetin, whereas the present study describes a medium that promotes callusing at the cut end of the stem and also the regeneration of shoot and root. The indiscriminate exploitation of *T. terrestris*, as a consequence of its being medicinally important, warrants discovery of quick methods for its replenishment and cultivation. The method reported in this paper can well facilitate the micropropagation of this plant.

ACKNOWLEDGEMENT

Authors thank the colleagues in plant tissue culture lab for their ungrudging help throughout this work.

LITERATURE CITED

Ali, G., M. Purohit, M.H. Mughal, M. Iqbal and P.S. Srivastava. 1996. A rapid protocol for micropropagation of *Bacopa monniera* (L.) Wettst--an important medicinal plant. *Plant Tiss. Cult. Biotech.* **2**: 208-211.

Anand, R., G.K. Patnaik, D.K. Kulshreshtha and B.N. Dhawan. 1994. Activity of certain fractions of *Tribulus terrestris* fruits against experimentally induced urolithiasis in rats. *Ind. J. Exp. Biol.* **32**: 548-552.

Anonymous. 1948. The Wealth of India. **Vol. I.** Publication and Information Directorate, New Delhi, India, 521 pp.

Erhun, W.O. and A. Sofowora. 1986. Callus induction and detection of metabolites in *Tribulus terrestris* L. *J. Plant Physiol.* **123**: 181-186.

Gulati, A., S. Bharel, M.Z. Abdin, S.K. Jain and P.S. Srivastava. 1996. *In vitro* micropropagation and flowering in *Artemisia annua*. *J. Biochem. Biotech.* **5**: 31-35.

Gunay, A.L. and P.S. Rao. 1978. *In vitro* plant regeneration from hypocotyl and cotyledon explants of red pepper (*Capsicum*). *Plant Sci. Lett.* **11**: 365-372.

Huang, L.C. and T. Murashige. 1976. Plant tissue culture media: major constituents, their preparation and some application. *Tiss. Cult. Associat. Mann.* **3**: 539-548.

Jaiswal, V.S., P. Narayan and M. Lal. 1989. Micropropagation of *Adhatoda vasica* Ness through nodal segment culture. *In* Tissue Culture and Biotechnology of Medicinal and Aromatic Plants. A.K. Kukreja, A.K. Mathur, P.S. Ahuja and R.S. Thakur (eds.). CIMAP, Lucknow, India, pp. 7-11.

Kapoor, L.D. 1990. Handbook of Ayurvedic Medicinal Plants. C.R.C. Press Inc., Boca Raton, 416 pp.

Lauzer, D., G. Laublin and M. Cappadocia. 1992. *In vitro* propagation and cytology of wild yams, *Dioscorea abyssinica* Hoch. and *D. mangenotiana* Miege. *Plant Cell. Tiss. Org. Cult.* **28**: 215-223.

Mohan, V., M. Purohit and P.S. Srivastava. 1995. *In vitro* micropropagation of *Moringa pterygosperma*. *Phytomorphology* **45**: 253-261.

Murashige, T. 1974. Plant propagation through tissue cultures. *Annu. Rev. Plant Physiol.* **25**: 135-166.

Murashige, T. 1984. Parameters in regenerating plants *in vitro*. *In* Plant Cell Tissue and Cell Culture Application to Crop Improvement. Czech. Acad. Sci. Prague, pp. 23-31.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant.* **15**: 473-497.

Purohit, M., D. Pande, A. Datta and P.S. Srivastava. 1995. Enhanced xanthotoxin content in regeneranting cultures of *Ammi majus* and micropropagation. *Planta Med.* **61**: 481-482.

Quraishi, A., V. Koche and S.K. Mishra. 1996. *In vitro* micropropagation from nodal segments of *Cleistanthus collinus*. *Plant Cell Tiss. Org. Cult.* **45**: 87-91.

Samantaray, S., G.R. Rout and P. Das. 1995. An *in vitro* study on organogenesis in *Trema orientalis* (Blume) Linn. *Plant Sci.* **105**: 87-94.

Sim, G.E., C.G. Goh. and C.S. Loh. 1990. Micropropagation of *Citrus nitis* Blaneo multiple bud formation from shoot and root explants in the presence of 6-benzyleaminopurine. *Plant Sci.* **59**: 203-210.

Skoog, F. and C.O. Miller. 1957. Chemical regulation of growth and organ formation in plant tissue cultured *in vitro*. *Symp. Soc. Exp. Biol.* **11**: 118-131.

Srivastava, P.S., M. Purohit, D. Pande and A. Datta. 1993. Phenotypic variation and alkaloid content in the androgenic plantlets of *Datura innoxia*. *Phytomorphology* **43**: 209-216.

Thorpe, T.A. 1980. Organogenesis *in vitro*. Structural physiological and biochemical aspects. *Int. Rev. Cytol.* **11**: 71-77.

Received December 11, 1996
Accepted June 10, 1997