International Journal of Plant Developmental Biology ©2008 Global Science Books

Somatic Embryogenesis and Plant Regeneration from Nodal Explants in *Psoralea corylifolia* L.

Mohd Faisal* • **Anwar Shahzad** • **Mohammad Anis****

Plant Biotechnology Laboratory, Department of Botany, Aligarh Muslim University, Aligarh-202 002, India

Corresponding authors: * **faisalm15@yahoo.com** ** **ansim1@rediffmail.com**

ABSTRACT

The development of efficient *in vitro* regeneration systems are needed to facilitate the application of recombinant DNA technology to the improvement of crop germplasm. In the present study a simple, rapid and effective system to regenerate *Psoralea corylifolia* plants via direct somatic embryogenesis from nodal segments has been established. The embryogenic cells proliferated, formed somatic embryos, and were subsequently converted into normal plantlets under optimized culture conditions. The frequency of somatic embryogenesis was strongly influenced by the concentration of thidiazuron (TDZ) in the medium. The highest frequency (82%) of somatic embryogenesis was observed on Murashige and Skoog (MS) medium containing 16.0 µM TDZ. The somatic embryos when transferred to plant growth regulator-free MS basal medium, developed further to heart shaped, torpedo and cotyledonary stages within 2 weeks. Conversion of somatic embryos into plantlets was achieved by isolating somatic embryos with distinct cotyledons and transferring them onto half-strength MS medium containing 1.0 µM gibberellic acid (GA_3). Subsequently, the regenerated plantlets were successfully established in *ex vitro* condition with 90% survival. This is the first report on *in vitro* regeneration via direct somatic embryogenesis of *P. corylifolia*.

Keywords: *in vitro*, medicinal plant, plant regeneration, thidiazuron
Abbreviations: **GA_3**, gibberellic acid; **MS**, Murashige and Skoog medium; **TDZ**, thidiazuron

INTRODUCTION

Psoralea corylifolia L. (Fabaceae), commonly known as 'babchi', is an endangered medicinal plant distributed in the tropical region of the world (Anonymous 1988). The plant is used in indigenous system of medicine as a laxative, aphrodisiac, anthelmintic, diuretic and diaphoretic in febrile conditions. It is specially recommended in the treatments of leucoderma, leprosy psoriasis and inflammatory diseases. The plant contains important principle furanocoumarin compounds, psoralen and isopsoralen which are being investigated against several diseases including AIDS (Bhattacharji 1998; Zhou *et al*. 2000). The one-seeded fruits are highly regarded as an aphrodisiac and tonic to the genital organs (Chopra *et al*. 1986). The plant is being used by many Indian pharmaceutical industries for the production of drugs (Research Drugs and Pharmaceuticals (Gujarat) Pvt. Ltd., Ahmadabad, India, Pioneer Enterprise, Mumbai, India and Dr. J. R. K's Siddha Research and Pharmaceuticals Pvt. Ltd., Chennai, India).

P. corylifolia is a seed propagated species, however, the germination percentage is very low (5-7%) (Chand and Sahrawat 2002). The low percentage of seed germination coupled with non-judicious wild collection for pharmaceuticals pose a serious threat to its existence in the nature. Tissue culture and *in vitro* plant regeneration system provide an alternative means for mass proliferation and *ex situ* conservation of endangered plant species. A few studies on plant regeneration in the genus *Psoralea* have been conducted, including plant regeneration via organogenesis (Saxena *et al*. 1998; Faisal and Anis 2006; Baskaran and Jayabalan 2008) and indirect somatic embryogenesis (Chand and Sahrawat 2002). However, there is no report on plant regeneration via direct somatic embryogenesis in *P. corylifolia*. Direct somatic embryogenesis from predetermined tissues is the most desirable approach, because it appears to be associated with the cytological and genetic stability of regenerated plantlets. It is also useful as a transformation system for the improvement of agronomic traits as well as a model system for 'molecular farming' (Daniell 2004). Here, in the present report, we describe a protocol for direct somatic embryogenesis from nodal segments of *P. corylifolia* and successful field establishment of the acclimatized plants.

MATERIALS AND METHODS

Plant material and explant preparation

Young, healthy shoots of *P. corylifolia* were collected from a two-year-old plant grown in the Botany Department, Aligarh Muslim University, Aligarh. The shoot segments were washed under running tap water for 30 min and then soaked in 5% (v/v) detergent, Labolene (Qualigens, Mumbai, India), for 5 min. They were surface sterilized with 70% ethanol for 20 s followed by 0.1% mercuric chloride treatment for 3 min and finally rinsed thrice with sterile double distilled water. Shoot segments measuring 4-7 mm were cut into single node explants and inoculated vertically on sterile nutrient media.

Culture media and conditions

Murashige and Skoog (1962; MS) medium containing 3% (w/v) sucrose and 0.8% (w/v) agar were used in all the experiments. Plant growth regulators (PGRs) were added to the medium as specified below. The pH of the culture media was adjusted to 5.8 ± 0.1 before addition of agar and sterilized by autoclaving for 20 min at 1.1 kg/cm^2 pressure at 121°C. Cultures were maintained in the culture room at 25 ± 1°C under 50 µmol m^{-2} s^{-1} light provided by cool white fluorescent lamps (TL 20W, Philips, India) for a 16 h photoperiod regulated by a timer.

Somatic embryo induction and development

For induction of somatic embryos, the sterile nodal explants were

Received: 27 June, 2008. Accepted: 9 September, 2008.

Short Communication

cultured in test tubes on MS medium supplemented with thidiazuron (TDZ)] at different concentrations (0.0, 10.0, 11.0, 12.0, 13.0, 14.0 15.0, 16.0, 17.0, 18.0, 19.0, 20.0 µM). The frequency of somatic embryogenesis was examined after 6 weeks of culture. The embryogenic cultures were transferred to PGR-free MS basal medium for 2 weeks. Cultures were examined and photographed with a stereo-zoom microscope (Motic-SMZ143, Japan). The number of embryos reaching the cotyledonary stage was recorded after 2 weeks and their number was expressed as somatic embryos per explant.

Germination and conversion into plantlets

The cotyledonary-stage somatic embryos were transferred to half-strength MS supplemented with various concentrations of gibberellic acid (GA_3) (0.0, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5 µM). The frequency of somatic embryo germination was examined after 4 weeks of culture.

Acclimatization

In vitro-developed plantlets (5-6 cm shoot length) were taken out of culture tubes, washed under running tap water to remove agar, and transplanted into plastic pots containing sterile soilrite. Potted plantlets were covered with a transparent polythene membrane and kept in a culture room at 25 ± 2°C temperature and 16h/day illumination with cool fluorescent light (50 µmol m^{-2} s^{-1}) for 4 weeks and watered every three days with half-strength MS salt solution for two weeks. Polythene membranes were opened after two weeks in order to acclimatize plants to field conditions. After four weeks, the plants were transferred to pots containing normal soil and maintained in a greenhouse under normal day length conditions.

Statistical analysis

A minimum of 20 explants were used per treatment and one explant was cultured per test tube. The experiments were repeated thrice. The data were collected after 6 weeks for embryogenesis and 4 weeks for germination experiments. The results were analyzed statistically using SPSS version 12 (SPSS Inc., Chicago, IL, USA). The significance of differences among means was analyzed using Duncan's multiple range test at P = 0.05.

RESULTS AND DISCUSSION

Nodal explants of *P. corylifolia* cultured on MS basal medium devoid of TDZ failed to give any response. The medium containing lower concentrations of TDZ (0.5-5.0 µM) induced multiple shoots (Faisal and Anis 2006) while in the present study, a higher concentration (10-20 µM) completely switched the regeneration pathway by inducing a cluster of well formed somatic embryos instead of shoot formation. The TDZ dose-dependent morphogenic response of nodal explants of *P. corylifolia* is a unique finding which clearly reaffirms the efficacy of TDZ for eliciting both organogenesis as well as embryogenesis. The redirection of development from shoot organogenesis to somatic embryogenesis at a higher concentration of TDZ (10-20 µM) may have occurred due to an optimum growth regulator balance within the tissue or as a result of increased stress imposed by a high TDZ concentration (Mithila *et al*. 2003). In earlier studies, it was documented that TDZ induced regeneration is accompanied by an accumulation of ABA, proline, and specific ions (Murthy *et al*. 1996, 1998; Murch *et al*. 1999). However, in chickpea cultures the addition of proline to the TDZ-containing medium stimulated somatic embryogenesis instead of organogenesis (Murthy *et al*. 1996). MS medium supplemented with TDZ at 10-20 µM induced a cluster of globular embryos within 4 weeks at the swollen region of the explants (**Figs. 1A, 1B**), and the frequency of embryogenesis recorded as the average number of embryos per explants was found highest at 16.0 µM TDZ (**Table 1**). The explants cultured under this regime showed embryogenesis in 82% of the cultured explants, with an average of 27.0 ± 1.15 embryos per responding explant. The percentage somatic embryogenesis was significantly and negatively affected by increasing the level of TDZ from 16 to 20 µM (P = 0.05).

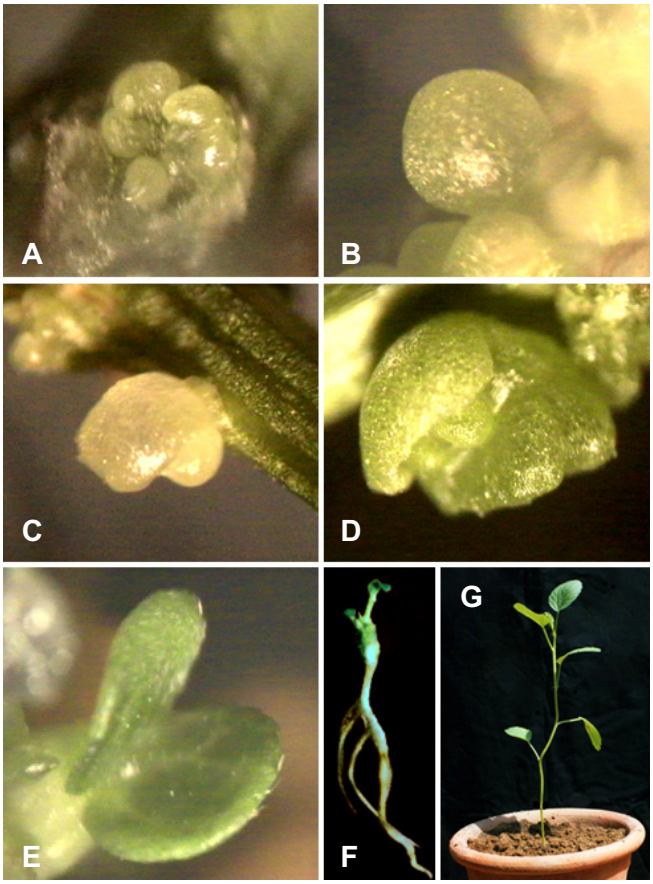

Fig. 1A-G Somatic embryogenesis and plant regeneration in *Psoralea corylifolia*. (**A, B**) Clusters of globular-stages somatic embryos developing directly from nodal segments; (**C**) Heart shaped embryos developing directly from explant; (**D, E**) Cotyledonary stages embryos; (**F**) Germination of somatic embryos; (**G**) Plantlet produced from somatic embryos.

Table 1 Effect of different concentrations of thidiazuron (TDZ) for direct somatic embryogenesis from nodal segment of *P. corylifolia* in MS medium after 6 weeks of culture.

Thidiazuron (µM)	Percentage of explants with somatic embryogenesis	Mean № of somatic embryos per responding explants ± S.E.
0.0	0.0 g	0.0 h
10.0	40.6 f	3.0 ± 0.14 g
11.0	45.3 e	5.5 ± 0.27 fg
12.0	59.0 c	7.1 ± 0.21 f
13.0	60.6 c	12.3 ± 1.45 cd
14.0	70.0b	15.3 ± 1.20 c
15.0	81.62 a	20.6 ± 1.33 b
16.0	82.0 a	27.0 ± 1.15 a
17.0	72.3 b	23.0 ± 1.16 b
18.0	69.6 bc	15.0 ± 1.01 c
19.0	51.3 d	10.7 ± 1.20 de
20.0	50.0 d	8.3 ± 0.67 ef

Data represents the mean ± SE of three repeated experiments. Means followed by different letter within columns are significantly different (P = 0.05) using Duncan's multiple range test.

When the clusters of embryos were transferred to MS basal medium without TDZ, growth of somatic embryos was stimulated until the cotyledonary stage (**Figs. 1C, 1D**) but did not germinate. When somatic embryos were maintained on the medium containing TDZ over a period of 6 months, the occurrence of fasciated embryos was noticed. This is a common phenomenon associated with the long-term use of TDZ (Huetteman and Preece 1993). Mature somatic embryos showing early cotyledonary stages were ra-

Table 2 Effect of gibberellic acid (GA_3) on germination of somatic embryos of *Psoralea corylifolia* in half-strength MS medium after 4 weeks of transfer.

Gibberellic acid (µM)	Number of embryos transferred	Number of embryos germinated	Percentage germination
0.0	60	12	20 f
0.1	60	32	53 d
0.5	60	45	75 b
1.0	60	54	90 a
1.5	60	40	67 c
2.0	60	34	56 d
2.5	60	14	23 e

Data represents the mean numbers of three repeated experiments. Means followed by different letter within columns are significantly different (P = 0.05) using Duncan's multiple range test.

pidly germinated (**Figs. 1E, 1F**) when they were transferred onto half-strength medium containing 0.1-2.5 µM GA_3 (**Table 2**). The major problem for propagation of a plant through embryogenesis has been the low conversion of somatic embryos into plantlets. In the present study, germination and conversion of over 90% of somatic embryos into plantlets occurred within 6 weeks of culture on half-strength MS medium containing 1.0 µM GA_3. A similar, beneficial effect of GA_3 on germination of somatic embryos has been demonstrated in *Santalum album* (Rai and McComb 2002), *Siberian ginseng* (Choi and Jeong 2002), *Gossypium hirsutum* (Kumria *et al*. 2003), and *Panax japonicus* (You *et al*. 2007).

The regenerated plantlets were transplanted to small plastic pots containing vermiculite and kept in a culture room for 4 weeks. They were irrigated once in 3 days with half-strength MS salts solution for two weeks and then individually transferred to pots containing soil, sand and farmyard manure (1:1:1) and reared in the greenhouse. About 90% of the regenerated plants survived following transfer from Soilrite to natural soil and no detectable variation with respect to morphology or growth characteristics was observed.

In conclusion, we have developed a direct somatic embryogenesis and plantlet regeneration system from nodal segments of *Psoralea corylifolia*. The protocol developed here for regeneration of *P. corylifolia* through direct somatic embryogenesis can possibly be used in future transformation and metabolic engineering studies.

ACKNOWLEDGEMENTS

Authors are thankful to the Department of Science and Technology, New Delhi for providing research assistance under DST-FIST Programme 2005 (Project No. SR/FST/LSI-085/2005 dated 20. 12. 2005) and awards of SERC-Fast Track Scheme for Young Scientist.

REFERENCES

Anonymous (1988) *The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products* (Vol IX), Council of Scientific and Industrial Research, New Delhi, 116-118

Baskaran P, Jayabalan N (2008) Effect of growth regulators on rapid micropropagation and psoralen production in *Psoralea corylifolia* L. *Acta Physiologiae Plantarum* **30**, 345-351

Bhattacharji SK (1998) *Handbook of Medicinal Plants*, Pointer Publisher, India, pp 287-288

Chand S, Sahrawat AK (2002) Somatic embryogenesis and plant regeneration from root segments of *Psoralea corylifolia* L., an endangered medicinal plant. *In Vitro Cellular and Developmental Biology - Plant* **38**, 33-38

Choi Y, Jeong J (2002) Dormancy induction of somatic embryos of *Siberian ginseng* by high sucrose concentrations enhances the conservation of hydrated artificial seeds and dehydration resistance. *Plant Cell Reports* **20**, 1112-1116

Chopra RN, Nayar SL, Chopra IC (1986) *Glossary of Indian Medicinal Plants*, Council of Scientific and Industrial Research, New Delhi, 330

Daniell H (2004) Chloroplast genetic engineering for improved agronomic traits and molecular farming, using various selection systems, World Congress. *In Vitro Biology* **40**, 18A

Faisal M, Anis M (2006) Thidiazuron induced high frequency axillary shoot multiplication in *Psoralea corylifolia*. *Biologia Plantarum* **50**, 437-440

Huetteman CA, Preece JE (1993) Thidiazuron: a potent cytokinin for woody plant tissue culture. *Plant Cell, Tissue and Organ Culture* **33**, 105-119

Kumria R, Sunnichan VG, Das DK, Gupta SK, Reddy VS, Bhatnagar RK, Leelavathi S (2003) High-frequency somatic embryo production and maturation into normal plants in cotton (*Gossypium hirsutum*) through metabolic stress. *Plant Cell Reports* **21**, 635-639

Mithila J, Hall JC, Victor JMR, Saxena PK (2003) Thidiazuron induces shoot organogenesis at low concentrations and somatic embryogenesis at high concentrations on leaf and petiole explants of African violet (*Saintpaulia ionantha* Wendl.). *Plant Cell Reports* **21**, 408-414

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-497

Murch SJ, Victor JMR, Krishnaraj S, Saxena PK (1999) The role of proline in thidiazuron-induced somatic embryogenesis of peanut. *In Vitro Cellular and Developmental Biology - Plant* **35**, 102-105

Murthy BNS, Murch SJ, Saxena PK (1998) Thidiazuron: a potent regulator of *in vitro* plant morphogenesis. *In Vitro Cellular and Developmental Biology - Plant* **34**, 267-275

Murthy BNS, Victor JMR, Singh RP, Fletcher RA, Saxena PK (1996) *In vitro* regeneration of chickpea (*Cicer arietinum* L): stimulation of direct organogenesis and somatic embryogenesis by thidiazuron. *Plant Growth Regulation* **19**, 233-240

Rai VR, McComb J (2002) Direct somatic embryogenesis from mature embryos of sandalwood. *Plant Cell, Tissue and Organ Culture* **69**, 65-70

Saxena C, Rout GR, Das P (1998) Micropropagation of *Psoralea corylifolia*. *Journal of Medicinal and Aromatic Plant Science* **20**, 15-18

You XL, Han JY Choi YE (2007) Plant regeneration via direct somatic embryogenesis in *Panax japonicus Plant Biotechnology Reports* **1**, 5-9

Zhou P, Takaishi Y, Duan H, Chen B, Honda G, Itoh M, Takedam Y, Kodzhimatovm OK, Lee KH (2000) Coumarins and bicoumarin from *Ferula sumbul*: anti-HIV activity and inhibition of cytokine release. *Phytochemistry* **53**, 689-697