Plant Biotechnology 26, 333–336 (2009)

Tissue Culture Note

In vitro regeneration of *Psoralea corylifolia* L. through callus cultures

P. Baskaran[a,]*, N. Jayabalan

Department of Plant Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu, India

* E-mail: baskaran_research@yahoo.co.in Tel: +972-542297215 Fax: +972-3-9669583

Received May 23, 2008; accepted January 13, 2009 (Edited by S. Ogita)

Abstract *In vitro* regeneration protocol was developed from hypocotyl-derived callus of *Psoralea corylifolia* L. Green compact nodular calli were induced from 3 day-old hypocotyl explants on Phillips and Collins (L2) medium containing 25 g l^{-1} sucrose, 7 g l^{-1} agar and supplemented with 10 μM NAA and 2 μM TDZ. Higher shoot regeneration (89.5 ± 1.18) was achieved in enriched L2 medium supplemented with 2 μM BA, 4 μM TDZ and 50 mg l^{-1} BVN. Regenerated shoots elongated on 1/2-enriched L2 medium containing 10 g l^{-1} sucrose, 7 g l^{-1} agar and 2 μM 2iP. Elongated shoots (45–55 mm in length) were exposed simultaneously 15.0 roots per shoot as well as hardened in moistened (1/8-L2 basal salt solution with 5 mM IBA and 100 mg l^{-1} BVN) soil mixture and vermiculite (3 : 1 v/v). The plants were subsequently established in the field. The higher survival percentage (100%) was achieved in winter season (September–December, 25–28°C). This system would be useful for mass propagation and germplasm conservation of *P. corylifolia*.

Key words: Callus culture, plant regeneration, *Psoralea corylifolia*.

Psoralea corylifolia L. (*Fabaceae*) is an endangered medicinal plant distributed widely in the tropical and sub-tropical regions. It is a rare and endangered medicinal plant of India, and an attractive annual herb, producing bluish purple flowers. The plant has been used in Indian and Chinese folk, Siddha, and Ayurvedic systems of medicine (Jain 1994). The plant contains major compounds such as coumarins, psoralen, isopsoralen, angelicin and daidzein. The plant exhibits antitumour, antibacterial, antifungal and antioxidative activities. It is also used in curing stomach ache, anthelmintic, diuretic and diaphoretic in febrile conditions (See Baskaran and Jayabalan 2007). Many Indian pharmaceutical industries have used *P. corylifolia* as a raw material in the production of medicines and Ayurvedic skin care soap (Baskaran and Jayabalan 2007). The wild population of this medicinally important plant exhibits high-mortality of the seedlings and also declined very fast due to indiscriminate and illegal collections, and destruction of its habitats. Therefore this species was included in the endangered list (Jain 1994). Hence, there is a need for the development of alternative plant regeneration tools for this valuable species.

Plant tissue culture techniques offer a viable tool for mass multiplication and germplasm conservation of rare and endangered medicinal plants for meeting the pharmaceutical needs (Sahoo and Chand 1998). The regeneration of callus has been successfully used for mass propagation and for obtaining useful variants in several plant species (Bouman and Klerk 2001). Therefore, the organogenesis technique would play an important role in germplasm conservation, genetic improvement and pharmaceutical needs of this plant. Although, plant regeneration from callus cultures of *P. corylifolia* has been published previously (Saxena et al. 1997), the present investigation describes rapid and greater number of shoot regeneration from hypocotyl explants of *P. corylifolia* by using enriched L2 medium, and different concentrations and combinations of plant growth regulators.

Mature seeds of *P. corylifolia* were washed thoroughly in tap water and surface sterilized with 2% (v/v) commercial detergent, Teepol (Reckitt Benckiser, India) for 2 min and 0.2% (w/v) $HgCl_2$ aqueous solution for 7 min. The seeds were then washed five times with sterile distilled water. About 50 seeds were soaked in 100 ml liquid L2 (Phillips and Collins 1979) medium and supplemented with 5 μM gibberellic acid (GA_3) for 12 h. Seeds were germinated on L2 medium (Phillips and Collins 1979) containing 25 g l^{-1} sucrose and 7 g l^{-1} agar

[a] Present address: Department of Vegetables Research, The Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel.

Abbreviations: BA, 6-Benzyladenine; BVN, Bavistin; 2,4-D, 2,4-Dichlorophenoxy acetic acid; GA_3, Gibberellic acid; IAA, Indole-3-acetic acid; IBA, Indole-3-butyric acid; 2iP, 2-isoPentenyladenine; KIN, Kinetin; NAA, α-Naphthaleneacetic acid; TDZ, Thidiazuron.

This article can be found at http://www.jspcmb.jp/

(Himedia, Mumbai, India) and maintained in 25 ± 2°C in darkness for 48 h. Thereafter they were placed in culture room conditions with 25 ± 2°C and 45–50 μmol $m^{-2} s^{-1}$ intensity of cool white fluorescent light with a 16-h light/8-h dark photoperiod. Three day-old hypocotyls (5–8 mm length) were inoculated onto L2 medium containing 10 μM 2,4-D and 2 μM BA; 10 μM 2,4-D and 2 μM TDZ; 10 μM NAA and 2 μM BA; and 10 μM NAA and 2 μM TDZ respectively for callus induction. Two-week-old organogenic calli (1.5 g fresh weight) were transferred to enriched L2 medium containing 15 g l^{-1} sucrose, 20% (v/v) watermelon juice (Anonymous 1992), 7 g l^{-1} agar and supplemented with 2, 4, 6, 8 and 10 μM BA or KIN or 2iP or TDZ, or 50, 100 and 150 mg l^{-1} BVN (carnbandazim powder, BASF, India) either individually or in combination for shoot regeneration. The regenerated shoots were transferred to 1/2-enriched L2 medium containing 10 g l^{-1} sucrose, 7 g l^{-1} agar and 2 μM 2iP for shoot elongation. Fresh watermelon cv. PKM1 (75 days from planting to maturity) was peeled and cut into 1 cm^3 cubes. The tissues were grinded in a turbo mixer (Maharaja Whiteline, India) for 5 min at room temperature (30°C) and filtered with a nylon mesh. The extract was then added to the medium. Media devoid of plant growth regulators and additives (watermelon juice and BVN) were used as control. All media were adjusted to pH 5.8 ± 0.1 with 0.1 N KOH before gelling with 7 g l^{-1} agar and then autoclaved at 121°C for 15 min. The cultures were maintained in the culture room conditions.

One week-old elongated shoots (45–55 mm) were dipped in 50 μM IBA for 15 min and were then transferred to plastic pots containing soil mixture and vermiculite (150 g/pot) (3 : 1 v/v) and irrigated with 50 ml of tap water used as control or 50 ml of 1/8-L2 basal salt solution supplemented with 5 mM IBA and 100 mg l^{-1} BVN respectively used to enhance the root formation and prevent the fungal contamination. The potted shoots were covered with transparent polythene bags for 2 weeks to prevent desiccation and they were maintained in the culture room conditions. The relative humidity was reduced gradually, and after 1 month plants were transferred to pots filled with 2 : 1 (v/v) mixture of soil and organic manure and maintained in a greenhouse. After 2 month, the plants were established to the field and then evaluated for survival percentage in summer (March–June, 36–45°C) and winter (September–December, 25–28°C) seasons.

The data were collected after 4 weeks for shoot regeneration and additional 2 weeks for rooting experiments. Experiments were set up in a randomized block design and each experiment was repeated three times with 25 explants per treatment in each experiment. The ANOVA was used to test the statistical significance, and the significance of differences among the means was carried out using DMRT (Gomez and Gomez 1976) at 5% level.

Various treatments of auxin and cytokinin combinations were used for organogenic callus production. Callus was initiated from the cut ends of the hypocotyl explants. The callus rating and morphogenic response were assessed after 2 weeks of culture. All explants produced callus from the treatments, while no callus was observed in control. Among the four different combinations, 10 μM 2,4-D and 2 μM BA, and 10 μM 2,4-D and 2 μM TDZ produced 2.0–3.0 g callus per explant approximately, and with yellowish green compact nodular callus; 10 μM NAA and 2 μM BA produced 2.5–4.0 g callus per explant approximately, and with whitish green compact callus, and 10 μM NAA and 2 μM TDZ produced 3.5–4.5 g callus per explant approximately, and with green compact nodular callus after 2 weeks of culture (Figure 1A). Auxin and cytokinin combination was most effective in organogenic callus production in *Vigna radiate* (Amutha et al. 2003). In our study, a quick, green and nodular callus was observed in TDZ treatment in combinations. Similarly, TDZ was most effective in nodular callus formation in *Echinacea purpurea* (Jones et al. 2007). In the present study, the callus growth was fast in the first 2 weeks culture, later slowed down, deep brown and finally declined. This was due to phenolic exudation. Therefore, we suggested, two week-old green compact nodular callus suitable for rapid shoot regeneration in *P. corylifolia*.

The calli differentiated into shoot bud in the treatments after a week. In our study, enriched media containing watermelon juice (Anonymous 1992) may also possibly influence shoot regeneration in *P. corylifolia*, as was also reported by Baskaran and Jayabalan (2008). Enriched L2 medium supplemented with 6 μM BA considerably increased number of shoots after 4 weeks culture (Table 1). However, BA did not produce shoots in the previous study (Saxena et al. 1997). Higher number of shoots (27.6 ± 0.92) with an average length of 34.5 ± 0.82 mm was obtained in 6 μM TDZ (Table 1, Figure 1B). In the present study, TDZ was effective in shoot regeneration in *P. corylifola*. Similarly, Faisal and Anis (2006) was also reported that effectiveness of TDZ in *P. corylifolia*. Increasing concentration of BA (8–10 μM), KIN (10 μM), 2iP (10 μM) and TDZ (8–10 μM) in the medium decreased number of shoots (Table 1). In our study, cytokinin combinations considerably increased number of shoots (Table 1). A similar result was also observed by Amutha et al. (2003). A combination of 2 μM BA and 6 μM TDZ was more effective in generating shoots (Table 1). Increasing concentration in combinations decreased shoots number and length (Table 1). Our finding was corroborated by Amutha et al. (2003). The present study

Table 1. Effect of BA, KIN, 2iP and TDZ on regeneration of shoots from green compact nodular callus of *Psoralea corylifolia*

Plant growth regulators (μM)				Number of regenerated shoots/explant	Shoot length (mm)
BA	KIN	2iP	TDZ		
4	—	—	—	10.4 ± 0.72m	13.6 ± 0.46l
6	—	—	—	23.6 ± 0.57g	29.0 ± 0.63cd
8	—	—	—	11.8 ± 0.36l	17.4 ± 0.57ij
10	—	—	—	5.2 ± 0.24q	12.2 ± 0.26lm
—	4	—	—	3.7 ± 0.18r	21.3 ± 0.22g
—	6	—	—	8.3 ± 0.32o	35.6 ± 0.42a
—	8	—	—	14.5 ± 0.47k	26.7 ± 0.64de
—	10	—	—	6.8 ± 0.26p	16.8 ± 0.27j
—	—	4	—	2.6 ± 0.18s	20.6 ± 0.38gh
—	—	6	—	6.4 ± 0.32p	31.2 ± 0.82c
—	—	8	—	9.5 ± 0.54n	24.5 ± 0.68f
—	—	10	—	4.1 ± 0.25r	15.7 ± 0.47jk
—	—	—	4	19.2 ± 0.67hi	16.9 ± 0.60j
—	—	—	6	27.6 ± 0.92f	34.5 ± 0.82ab
—	—	—	8	20.5 ± 0.64h	27.4 ± 0.75d
—	—	—	10	9.2 ± 0.25n	15.2 ± 0.56jk
2	—	—	6	43.2 ± 1.06a	21.1 ± 0.94g
4	—	—	6	34.6 ± 1.12c	17.8 ± 0.62ij
—	2	—	6	29.7 ± 1.02e	29.2 ± 0.86cd
—	4	—	6	37.6 ± 1.23b	20.4 ± 0.78gh
—	6	—	6	18.5 ± 0.62i	11.3 ± 0.25m
—	—	2	6	29.2 ± 1.08e	24.6 ± 0.84f
—	—	4	6	32.4 ± 1.14d	18.0 ± 0.42i
—	—	6	6	16.4 ± 0.94j	8.5 ± 0.24n

Data were recorded after 4 weeks of culture.
Values followed by different letters indicate significant difference between means ($p<0.05$); comparison by DMRT.

revealed that cytokinin (BA and TDZ) combination is essential for increasing number of shoots in *P. corylifolia*. However, cytokinins and BVN combination positively influenced shoot regeneration in *P. corylifolia*. Enriched L2 medium containing BVN produced shoots, it might possibly be due to "cytokinin-like" activity. Similar results were also observed by Tiwari et al. (2006) and Baskaran and Jayabalan (2008). Moreover, BVN was produced callus in *Daucus carota* (Tripathi and Ram 1982). A number of shoots considerably increased in 4 μM TDZ and 100 mg l^{-1} BVN (Table 2, Figure 1C). Maximum number of 89.5 ± 1.18 shoots was obtained in 2 μM BA, 4 μM TDZ and 50 mg l^{-1} BVN (Table 2, Figure 1D). Increasing concentration in combinations decreased shoots number and length (Table 2). Therefore, the present study suggested cytokinins (BA and TDZ) and BVN combination suitable for improving shoots number in *P. corylifolia*.

The elongation of 45–55 mm shoot length was attained in 1/2-enriched L2 medium supplemented with 2 μM 2iP after one week cultures (Figure 1E). Cytokinins were shown to be the most critical growth regulator for shoot elongation in medicinal plants (Sharma et al. 1993; Baskaran and Jayabalan 2005). Elongated shoots produced rooting within one week after transferring into the soil : vermiculite (3 : 1, v/v) mixture and irrigating with 50 ml of 1/8-L2 basal salt solution supplemented with 5 mM IBA and 100 mg l^{-1} BVN.

Table 2. Effect of BA, TDZ and BVN on regeneration of shoots from green compact nodular callus of *Psoralea corylifolia*

BA (μM)	TDZ (μM)	BVN (mg l^{-1})	Number of regenerated shoots/explant	Shoot length (mm)
—	—	50	4.2 ± 0.26m	8.4 ± 0.12j
—	—	100	9.6 ± 0.42l	12.8 ± 0.26hi
—	—	150	3.7 ± 0.21n	6.2 ± 0.18l
4	—	50	18.4 ± 0.68k	36.3 ± 0.54a
6	—	50	23.5 ± 0.84j	25.8 ± 0.32d
4	—	100	37.5 ± 1.02f	22.6 ± 0.37e
6	—	100	26.2 ± 0.53i	16.4 ± 0.28g
—	4	50	30.2 ± 0.72gh	34.2 ± 0.65ab
—	6	50	54.4 ± 1.06d	27.6 ± 0.58cd
—	4	100	63.6 ± 1.12c	29.4 ± 0.52c
—	6	100	52.0 ± 1.04e	18.7 ± 0.24f
2	4	50	89.5 ± 1.18a	23.5 ± 0.62de
2	4	100	70.4 ± 0.92b	17.0 ± 0.43fg
2	6	50	52.7 ± 0.68e	13.6 ± 0.26h
2	6	100	31.5 ± 0.37g	7.8 ± 0.18jk

Data were recorded after 4 weeks of culture.
Values followed by different letters indicate significant difference between means ($p<0.05$); comparison by DMRT.

An average number of roots (15.0) per shoot with a 65–85 mm length were recorded after two weeks of plantation. The addition of BVN in the 1/8-L2 basal salt solution, prevented fungal contamination of the moist soil mixture and favored shoot growth. An average number of roots (4.0) per shoot with a 25–35 mm length were observed in the water-irrigated control that was infected by fungal contamination. The plantlets were

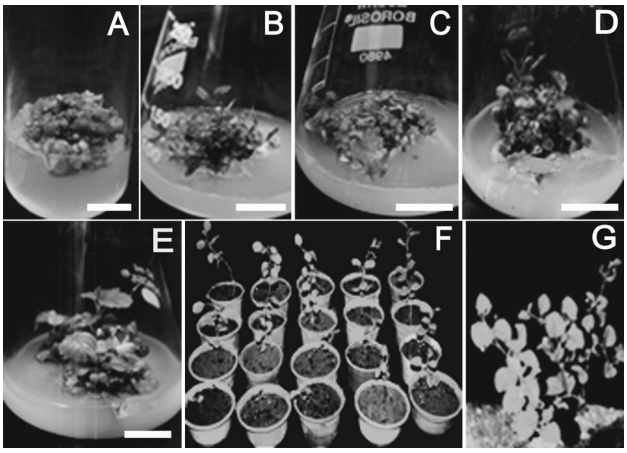

Figure 1. *In vitro* regeneration from hypocotyl explants of *P. corylifolia*. (A) Green compact nodular callus on L2 medium supplemented with 10 μM NAA and 2 μM TDZ. (B) Shoot regeneration on enriched L2 medium supplemented with 6 μM TDZ. (C) Shoot regeneration on enriched L2 medium supplemented with 4 μM TDZ and 100 mg l^{-1} BVN. (D) Shoot regeneration on enriched L2 medium supplemented with 2 μM BA, 4 μM TDZ and 50 mg l^{-1} BVN. (E) Elongation of shoots. (F) Regenerated plants in greenhouse. (G) Regenerated plants of *P. corylifolia* in the field. Bars = 1 cm.

transferred to mixture of soil and organic manure (2 : 1, v/v) and maintained in the greenhouse (Figure 1F). The regenerated plants were successfully established in the field (Figure 1G). About 200 plants were tested for the survival rate in different season. The survival rate during summer was 55–60% whereas in winter it was 100%. High temperatures (36–45°C) could be unfavorable for the establishment of plantlets in the field whereas low temperatures (25–28°C) during winter could be favorable for establishment. The study therefore suggests that for *P. corylifolia* hardened plants should be transferred to field only during winter season for the best survival rate.

In conclusion, the present study describes an effective method for shoot regeneration through callus culture from hypocotyl explants of *P. corylifolia* by using enriched L2 medium, different concentration and combination of growth regulators and BVN. This protocol will be helpful for mass propagation, conservation strategy, somaclonal variation and phytomedicine studies of *P. corylifolia*.

Acknowledgements

This work was supported by the Department of Science and Technology, India in the form of Senior Research Fellow. The authors are indebted to Aaron Zelcer (ARO, Israel) for his critical comments on this manuscript, and are grateful to Dhiraviyadas (Bharathidasan University, India) for his valuable assistance during seed collection.

References

Amutha S, Ganapathi A, Muruganantham M (2003) *In vitro* organogenesis and plant formation in *Vigna radiata* (L.) Wilczek. *Plant Cell Tiss Org Cult* 72: 203–207

Anonymous (1992) The wealth of India—a dictionary of Indian raw materials and industrial products. Publication and Information Directorate, New Delhi, India

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

Baskaran P, Jayabalan N (2007) Rapid micropropagation of *Psoralea corylifolia* L. using nodal explants cultured in organic additive-supplemented medium. *J Hort Sci Biotechnol* 82: 908–913

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

Bouman H, De Klerk GJ (2001) Measurement of the extent of somaclonal variation in Begonia plants regenerated under various conditions. Comparison of three assays. *Theor Appl Genet* 102: 111–117

Faisal M, Anis M (2006) Thidiazuron induced high frequency axillary shoot multiplication in *Psoralea corylifolia*. *Biol Plant* 50: 437–440

Jain SK (1994) Ethanobotany and research in medicinal plant in India. *Ethanobotany Sear for New Drugs* 185: 153–168

Gomez KA, Gomez KA (1976) *Statistical procedures for agricultural research with emphasis on Rice*. Philippines International Rice Research Institute, Los Banos.

Jones MPA, Yi Z, Murch SJ, Saxena PK (2007) Thidiazuron-induced regeneration of *Echinacea purpurea* L.: Micropropagation in solid and liquid culture systems. *Plant Cell Rep* 26: 13–19

Phillips GC, Collins GB (1979) *In vitro* tissue culture of selected legumes and plant regeneration from callus of red clover. *Crop Sci* 19: 59–64

Sahoo Y, Chand PK (1998) Micropropagation of *Vitex negundo* L. a woody aromatic medicinal shrub, through high frequency axillary shoot proliferation. *Plant Cell Rep* 18: 301–307

Saxena C, Palai SK, Samantaray S, Rout GR, Das P (1997) Plant regeneration from callus cultures of *Psoralea corylifolia* L. *Plant Growth Regul* 22: 13–17

Sharma N, Chandel KPS, Paul A (1993) *In vitro* propagation of *Gentiana kurroo*: an indigenous threatened plant of medicinal importance. *Plant Cell Tiss Organ Cult* 34: 307–309

Tiwari V, Tiwari KN, Singh BD (2006) Shoot bud regeneration from different explants of *Bacopa monnieri* (L.) Wettst. by trimethoprim and bavistin. *Plant Cell Rep* 25: 629–635

Tripathi RK, Ram S (1982) Induction of growth and differentiation of carrot callus cultures by carbendazim and benzimidozole. *Ind J Exp Biol* 20: 674–677