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In vitro Plant Regeneration from Cotyledonary Node of *Psoralea corylifolia* L.

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Abstract

Cotyledonary node of *Psoralea corylifolia* L. gave rise to multiple shoots when cultured on MS medium supplemented with different concentrations of BAP and Kn. The highest rate of shoot multiplication was obtained in MS containing 2.22 µM BAP. The regenerated shootlets were rooted on MS basal medium with different concentrations of IBA. The maximum number of roots was produced on the medium containing 4.92 µM of IBA. The plantlets, thus developed were hardened and successfully established in soil. Tissue culture raised plants exhibited normal growth, flowering and pod setting.

Introduction

Psoralea corylifolia L. is an important medicinal plant belonging to Leguminosae, distributed in the tropical and subtropical regions (Jain 1994). It is used as laxative, aphrodisiac, anthelmintic, diuretic and diaphoretic in febrile conditions. It has been specially recommended in the treatment of leucoderma, leprosy, psoriasis and inflammatory diseases of the skin and prescribed both for oral administration and external application in the form of a paste or ointment (Anon. 1998). Pharmaceutical companies largely depend upon materials procured from naturally occurring stands causing rapid depletion of this important source of medicinal herb. Hence, it has become imperative to establish a suitable protocol to generate enough matrials to ensure its supply for pharmaceutical industries without further depopulating this species. Limited tissue culture work has been done on *Psoralea corylifolia* (Saxena et al. 1997; Jeyakumar and Jayabalan 2000). This paper describes an efficient and rapid propagation method of *Psoralea corylifolia* using cotyledonary node explants.

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Materials and Methods

Plants of *Psoralea corylifolia* L. were collected from wild stands and maintained in the Botanical Garden of Bharathidasan University, Tamil Nadu. Uniform and healthy seeds were washed in running tap water and then washed again thoroughly by adding a few drops of Tween-80. Thereafter they were surface sterilized in a 0.1 % mercuric chloride for 5 min followed by rinsing them five times in sterile water. The clean seeds were germinated in MS basal medium. The pH of the medium was adjusted to 5.8 with 0.1 N NaOH before autoclaving at 1.06 kg/cm^{-2} and 121_C for 15 min. All culture media contained 3 % sucrose (w/v) and solidified with 0.8 % agar.

Cotyledonary node explants obtained from 20-day-old seedlings. Explants were aseptically cultured on MS basal medium supplemented with different concentrations of BAP and Kn. Shoots were excised from shoot clumps and transferred to MS fortified with various concentrations of IBA for root induction. The rooted shoots were removed from the culture medium. After removing traces of agar sticking to the surface of their roots with distilled water, they were transferred to plastic cups containing a mixture of garden soil, vermiculite and sand (1 : 1 : 1). Potted seedlings were grown under laboratory conditions of regulated humidity and temperature for two weeks and watered once every three days. The plants were kept under shade for four weeks and then placed under full sunlight.

Data were scored after 20 and 30 days for recording multiple shoot induction and rooting fequency, respectively. Only data which showed some advantageous effect were included in the tables and have been presented in mean ± S.E. of 24 explants per treatment and repeated three times. Mean values with the same superscirpt were not significantly different (p = 0.05 %) according to Duncan's Multiple Range Test (DMRT).

Results and Discussion

The effect of BAP and Kn on shoot multiplication from cotyledonary nodes is shown in Table 1. The explants showed shoot initiation after seven days (Fig. A). The number of shoots in medium with BAP were greater than those observed in the medium supplemented with Kn. In the medium containing 2.22 µM BAP the number of shoots were 7.0 per explant (Table 1, Fig. B). When BAP concentration was increased above 2.22 µM, the rate of shoot multiplication was reduced. Similar results have already been reported for

many medicinal plants such as *Malus sylvertris* (Hutchinson 1982), *Morus nigra* (Yadav et al. 1990), *Zingiber officinale* (Balachandran et al. 1966), *Piper* spp. (Bhat et al. 1995), *Plumbago zeylanica* (Susmita and Debata 1998) and *Houttuynia cordata* (Handique and Pranjal 1999).

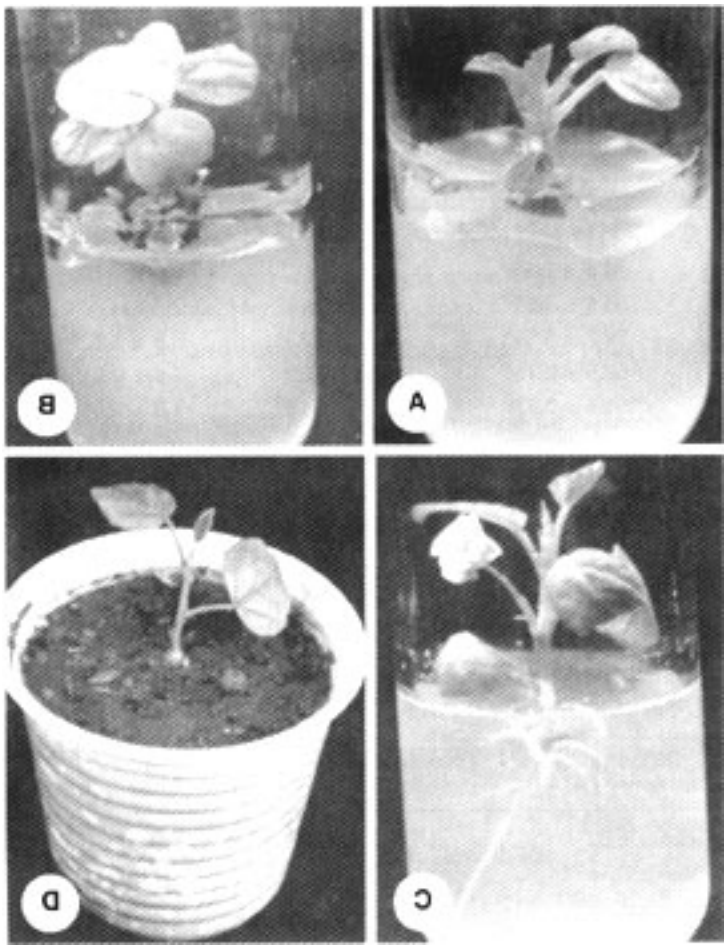

Fig. A. Shoot initiation on MS fortified with BAP (2.22 µM) after seven days. B. Shoot proliferation on MS supplemented with BAP (2.22 µM) after 30 days. C. Micro-propagated shoot rooted on 4.92 µM of IBA after 20 days. D. Plantlet established in plastic cups after 15 days.

Of the different concentrations of IBA (1.23 - 7.38 µM) tested 4.92 proved to be most suitable for root induction with 6.4 ± 1.0 roots per explant. The average root length being 4.9 ± 1.2 cm (Table 2, Fig. C). Similar effects of IBA were also observed in *Cephaelis ipecacuanha* (Jha and Jha 1989), *Plantago ovata* (Wakhlu and Barna 1989), *Rheum emodi* (Lal and Ahuja 1989), *Sesbania*

acculeata (Bensal and Pandey 1993), Pigeon pea (Sivaprakash et al. 1994), *Vitex negundo* (Thiruvengadam and Jayabalan 2000) and *Peganum harmala* (Raman and Jaiwal 2000). This is in contrast with the results of Madhavan and Balu (1995) on *Wedelia chinensis*, wherein IBA promoted formation of multiple shoots.

Table 1. Effect of BAP and Kn on shoot regeneration from cotyledonary node explants of *Psoralea corylifolia*.

Plant growth regulators (µM)	Regeneration responses (%)	No. of shoots/ explant	Shoot length (cm)
BAP			
0.44	34.8 ± 2.85^{d}	1.2 ± 0.02^{de}	2.0 ± 0.10^{c}
1.11	55.6 ± 2.85^{bc}	3.2 ± 0.40^{bc}	2.7 ± 0.7^{b}
2.22	79.7 ± 5.59^{a}	7.0 ± 0.32^{a}	5.8 ± 1.40^{a}
3.33	68.5 ± 2.16^{ab}	4.0 ± 0.29^{b}	2.4 ± 0.01^{bc}
4.44	61.4 ± 2.77^{b}	1.5 ± 0.16^{d}	1.8 ± 0.08^{cd}
Kn			
0.46	28.5 ± 6.04^{d}	1.0 ± 0.02d	2.9 ± 0.12^{c}
1.16	47.4 ± 3.79^{bc}	1.4 ± 0.10bc	3.2 ± 0.07^{b}
2.32	65.3 ± 1.24^{a}	2.0 ± 0.40a	4.4 ± 1.20^{a}
3.48	50.6 ± 2.01^{b}	1.6 ± 0.47b	3.0 ± 0.04^{bc}
4.64	41.4 ± 1.46^{c}	1.0 ± 0.01d	2.2 ± 0.05^{d}

Table 2. Effect of IBA on *in vitro* rooting of shoots after 20 days of culture.

IBA conc. (µM)	Rooting response (%)	No. of roots/ explant	Root length (cm)
1.23	35.6 ± 6.02^{ef}	1.2 ± 0.60^{d}	1.8 ± 0.60^{de}
2.46	56.6 ± 3.16^{cd}	3.0 ± 1.00^{c}	2.4 ± 1.20^{d}
3.69	79.6 ± 3.21^{ab}	4.7 ± 0.80^{b}	3.6 ± 1.00^{bc}
4.92	83.0 ± 2.82^{a}	6.4 ± 1.00^{a}	4.9 ± 1.20^{a}
6.19	63.3 ± 4.16^{c}	5.4 ± 1.20^{ab}	4.0 ± 1.40^{b}
7.42	36.0 ± 2.08^{e}	2.2 ± 0.60^{cd}	3.2 ± 0.53^{c}

Success of transplantation was 85 % when plantlets were sufficiently healthy with new growth, they were subsequently transferred to larger pots (Fig. D) and gradually acclimated to outdoor conditions. The protocol reported here is reproducible; it has a potential for being utilized to conserve the germplasm and allowing at the same time a large scale micropropagtion of this important medicinal plant.

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