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Research Article

IN-VITRO PROPAGATION OF AN ENDANGERED MEDICINAL PLANT *PSORALEA CORYLIFOLIA* LINN

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ABSTRACT

Objective: Here, we established the protocol for plant regeneration of *Psoralea corylifolia* via *In-vitro* micro propagation. Methods: The Apical meristems was used as the explants cultured on Murashige and Skoog (1962) medium (MS) supplemented different concentrations and combinations of plant growth regulators, 6-Benzylaminopurine (BAP), Kinetin (Kn), 1-Naphthaleneacetic acid (NAA) and B5 vitamins + 2 mg/ltr. Glycine (MBG). Results: Highest Shoot regeneration (95%) results were obtained on MS medium containing BAP (12 µM) with NAA (10.0 µM) and Kn (15.0 µM) generating shoots (6.12 shoots). BAP 12 µM found to be best for shoot multiplication. All the micro-shoots produced normal roots within two weeks of culture on the basic MS medium supplemented with auxin, viz. IAA, IBA or NAA. Maximum of 95% shoots were rooted with an average of 6.8 roots per shoot and average length of 7.11cm on MS medium supplemented with 2.5 µM IBA. Plantlets were transferred to pots where they grew well, attained maturity and set viable seeds. Conclusion: The micro propagation protocol reported here was characterized with a rapid proliferation of shoots, easy rooting of the micro-shoots and the plantlets were easily acclimatized to the external environment and undergoing normal physiological development.

Keywords:

INTRODUCTION

India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society whether directly as folk medicines or indirectly as pharmaceutical preparation of modern medicine [1]. The use of different parts of several medicinal parts to cure specific diseases has been in vogue from ancient times. *Psoralea corylifolia* Linn (Indian bread root) belonging to Fabaceae family is an endangered medicinal plant that is distributed throughout tropical and subtropical regions of the world. Its medicinal usage is reported in Indian pharmaceutical codex, Chinese, British and the American pharmacopoeias and in different traditional system of medicines such as Ayurveda, Unani and Siddha. *Psoralea corylifolia* grows as winter season weed. It is an erect annual herb, 30 -180 cm. Leaves are broadly elliptic, arranged in racemes. Flowers are yellow or bluish purple colour and Seeds are smooth, adhering to the pericarp, dark brown and elongated [2].

The plant contains major compounds such as coumarins, psoralen, isopsoralen, angelicin. Daidzein (4',7-dihydroxyisoflavone) and genistein (4',5,7 trihydroxyisoflavone) are the major bioactive isoflavones reported from *P. corylifolia* [3,4].

The plant exhibits antitumor, antibacterial, antifungal and antioxidative activities. It is also used in curing stomach ache, anthelmintic, diuretic, diabetes and diaphoretic in febrile conditions [5]. Many Indian pharmaceutical industries have used *P. corylifolia* as a raw material in the production of medicines and Ayurvedic skin care soap [5]. The seed oil is extremely beneficial, externally in numerous skin ailments. **:** Its oil has a specific irritant action on the skin and mucous membrane. On applying the oil on leucoderma, the skin becomes reddish and occasionally blisters may occur.

It stimulates the heart and circulatory system, it is used in cardiac failure and edema produced by it. *P. corylifolia is* useful in indigestion, and constipation. It is useful in all types of worms, especially round worms. As a liver stimulant it is useful for piles. It used in coughs and asthma since it reduces kapha by its katu rasa and ushna virya.

Consumer demand for high quality medicinal herbs is increasing at a slow, but steady, rate. Many of these herbs are harvested exclusively from stagnant to declining wild populations. One of the possible methods of protection of endangered taxon is multiplying and conservation of plants with help in vitro cultures. Biotechnological approaches, specifically plant tissue culture plays a vital role in search for alternatives to production of desirable medicinal compounds from plants.

The gradual decline in the population of *Psoralea corylifolia* demand launching of conservation effort so as to ensure continuous and ample supply by establishing a balanced cycle of harvest and renewal. Such conservation efforts would ensure continuous and ample supply of this valuable material which is in great demand by the pharmaceutical industry.

MATERIAL AND METHODS

Sterilization of explants

Shoot tip or Apical meristems that are of 0.5mm in length isolated from growing tips of current shoots of *Psoralea corylifolia* were used in the experiment as explants. Explants were washed under running tap water to remove dust particles for 30 min, and treated with liquid detergent for 10 min, and rinsed three times with distilled water. Then explant treated with an antifungal agent (Bavistin) for 1 hours and the again rinsed three times with distilled water. Further, sterilization treatments were done under a laminar-flow chamber. The explants were then disinfected with 0.1% (w/v) mercuric chloride for 3 minutes under aseptic conditions. After this these explants were then thoroughly washed 3-4 times with sterilized double distilled water to remove the traces of mercuric chloride now the explants is ready for inoculation on required medium. Shoot tip prepared from twenty day old in vitro raised seedlings were also used as explant. Shoot tip was cut from the top measuring 1-2 cm inside the laminar air flow chamber.

Inoculation in culture medium and Shoot Proliferation

Explants were cultured on MS basal medium supplemented with different concentrations and combinations of plant growth regulators, 6-Benzylaminopurine BAP: 1.0 - 20.0 µM), Kinetin (Kn: 2.5- 20.0 µM), 1-Naphthaleneacetic acid (NAA: 3.0- 20.0 µM) and B5 vitamins + 2mg / ltr. Glycine (MBG) formulation for shoot proliferation and multiplication. All cultures were incubated under 16h photoperiod with light intensity of 55µmolm^{-2}s^{-2} provided by cool white fluorescent lamps (Phillips, India) at 25 ± 2°C. All the cultures were transferred to fresh medium after 2-3 week duration. The frequency and number of shoots formed were evaluated after 6 weeks of inoculation. Morphological changes were recorded on the basis of visual observations at 3-week intervals.

Rooting of shoots and transfer of plantlets to soil

Shoots of 2-3 cm in height with two or three leaflets derived from cultures were transferred to MS medium containing 1 mg/l NAA + 20g sucrose or IAA or IBA or 200mg activated charcoal separately for rooting. The rooted shoots were transferred to MS medium for further elongation of the roots. The rooted plants were washed with water to remove media then transferred to pots containing autoclaved vermiculite soil and sand (1:2:1), and covered with polyethylene bags for one week to maintain high humidity and subsequently exposed to low air humidity for increasing period and finally polyethylene bags were removed. These hardened plants then transferred to the greenhouse [6,7].

RESULT AND DISCUSSION

After 2-3 weeks from culture initiation, shoots appeared and increased on subsequent sub-culturing (Fig. 1). MS medium without growth regulator did not initiate shoot differentiation. Number of shoots per explants among different concentrations and combinations of growth regulators were significantly different. All the concentrations of BAP, NAA and Kn alone facilitated the shoot differentiation (Fig. 2). Among the various combinations of BAP, Kn and NAA, highest Shoot regeneration (95%) results were obtained on MS medium containing BAP (12 µM) with NAA (10.0 µM) and Kn (15.0 µM) generating shoots (6.12 shoots) (Table 1). When BAP concentration was increased above 15 µM, the rate of shoot multiplication was reduced. Increasing concentrations of Kin (>20 µM) and NAA (>15 µM) decreased shoot number and length. The synergistic effect of auxin and cytokinin has been demonstrated in several medicinal plants, viz. *Santolina canescens* [8], *Rauvolfia tetraphylla* [9], *Bupleurum fruticosum* [10] and *Rotula aquatic* [11]. In accordance with these reports, the present investigation also exemplifies the positive modification of shoot induction efficiency by an auxin in combination with cytokinin. However, when NAA was replaced with IAA, a decrease in induction and multiplication of shoots was noticed.

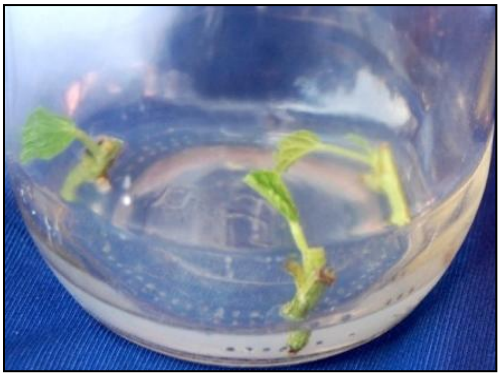

Figure 1: Shoot initiation through apical meristem of *P. corylifolia*

Figure 2: Shoots elongation of *P. orylifolia*

Sub-culturing for shoot multiplication

For multiplication shoots were subcultured on MS basal medium supplemented with different concentrations BAP (BAP: 1.0 - 20.0 µM).Among the various concentration, BAP 12 µM found to be best for shoot multiplication. MS medium containing BAP (12 µM) within 4 weeks, generates shoot 25 ±4 percent of length 3.1cm (Fig - 3, 4). Increasing the concentration of BAP more than 12 µM produced stunted shoots. Cytokinins have been shown to be most critical growth regulator for shoot multiplication in many medicinal plant sp. Like *Gentiana kurroo* [12]. So in present study medium with BAP acted as trigger for initiating multiplication of shoot bud meristem (Table 2).

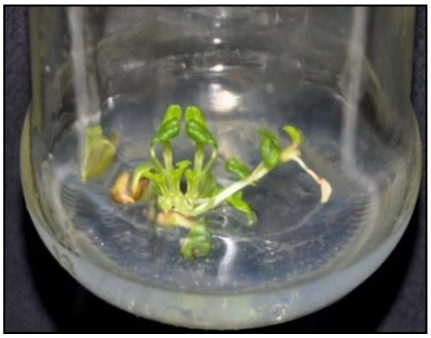

Figure 3: Shoot multiplication *P. corylifolia*

Figure 4: Shoot multiplication *P. corylifolia*

Rooting of shoots

The regenerated shoots were excised and transferred to MS basal medium supplemented with auxins, viz. IAA, IBA or NAA, for root formation. Maximum of 95% shoots were rooted with an average of 6.8 roots per shoot and average length of 7.11cm on MS medium suplimented with 2.5 µM IBA (Table 3, Fig- 5). The presence of auxins at lower concentration facilitated better root formation. MS medium supplemented with IBA was found superior to IAA and NAA with respect to the induction of roots. Superiority of IBA over other

auxins in root formation has also been reported in other plant species such as *Cunila galoides* [13] and *Clitoria ternatea* [14].

Figure 5: Rooting of *P. corylifolia*

Hardening

Plantlets were transferred to pots where they grew well, attained maturity and set viable seeds **(**Fig 8). The regenerated plants were successfully established in the field. 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 [15]. Organized development leading to plantlet regeneration via embryogenesis has successfully been achieved in many plant species through judicious selection of the inoculum, proper choice of the medium, including the growth-active substances, and the control of the physical environment [16-20].

Figure 8: Plantlets of *P. corylifolia* were transferred to pots

Tables 1: Effect of plant growth regulators on shoot regeneration

MS + Auxins / cytokinin (ì*M*)			Shoot regeneration (%)	No. of shoots
BAP	**Kn**	**NAA**		
1.0	-	-	25	1.45 ± 1
3.0	-	-	40	2.6 ± 3
6.0	-	-	70	2.43 ± 2
9.0	-	-	85	4.51 ± 1
12.0	-	-	90	5.95 ± 4
15.0			82	4.12 ± 3
18.0	-	-	40	2.92 ± 1
	2.5	-	50	1.67 ± 1
	5.0	-	55	2.21 ± 3
	10.0	-	70	3.16 ± 2
	15.0	-	85	5.11 ± 1
	20.0	-	40	3.13 ± 1
-		3.0	60	1.33 ± 3
-		5.0	75	2.16 ± 3
-		10.0	85	3.17 ± 2
-		15.0	80	4.39 ± 2
-		20.0	70	3.12 ± 1
6.0	10.0	-	40	2.51± 2
9.0	10.0	-	70	3.1 ± 2
12.0	10.0	-	80	3.2 ± 3
15.0	10.0	-	85	4.12 ± 1
6.0	15.0	-	60	3.25 ± 1
9.0	15.0	-	80	6.13 ± 3
12.0	15.0	-	90	7.14 ± 1
15.0	15.0	-	85	6.23 ± 2
6.0	15.0	10.0	70	3.6 ± 3
9.0	15.0	10.0	83	4.9 ± 2
12.0	15.0	10.0	95	6.12± 2
15.0	15.0	10.0	85	5.5 ± 2
15.0	15.0	10.0	70	4.5 ± 2

Table 2: Effect of cytokinin (BAP) on multiple shoot formation

MS Medium + BAP (µM)	Culture with multiple shoot (%)	Number of shoots (cm)	Length of shoots (cm)
2.0	10 ± 3	1.2	1.2
4.0	15 ± 2	1.6	1.6
6.0	15 ±4	3.1	1.8
8.0	18 ± 2	4.9	1.9
10.0	20 ± 1	5.1	2.1
12.0	25 ± 4	5.8	3.1
15.0	20 ±4	6.1	2.5
18.0	10 ± 1	1.6	1.4

Table 3: Rooting response of *P. corylifolia* on MS medium supplemented with various concentrations of IAA, IBA, and NAA

MS + Auxins (ìM)			Percentage of rooting (%)	Number of roots per shoot	Root length (cm)
IAA	IBA	NAA			
3.0	-	-	80	5.4	6.65 ± 1
6.0	-	-	75	4.8	4.95 ± 3
9.0	-	-	71	2.3	3.92 ± 1
12.0	-	-	45	1.1	1.81± 2
-	2.5	-	95	6.8	7.11 ± 4
-	5.0	-	82	4.6	6.08± 2
-	7.5	-	63	3.5	4.05 ± 1
-	10.0	-	40	2.7	3.17± 2
-	-	3.0	90	5.6	5.06 ± 3
-	-	5.0	85	3.8	3.21 ± 1
-	-	8.0	65	2.1	1.14 ± 2
-	-	10.0	50	1.1	1.32 ± 1

CONCLUSION

The micropropagation protocol reported here was characterized with a rapid proliferation of shoots, easy rooting of the micro-shoots and the plantlets were easily acclimatized to the external environment and undergoing normal physiological development. This is highly advantageous for the production of uniform source of *Psoralea corylifolia* plants for a range of further biotechnological applications.

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