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Callus Induction and Organogenesis from Leaf Explants of *Psoralea corylifolia* Linn: An Endangered Medicinal Plant

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Abstract: A callus induction and plant regeneration protocol was developed from leaf explants of the *Psoralea corylifolia* Linn. Explants were cultured on MS medium supplemented with different plant growth regulators (PGRs) i.e. 2, 4-D, BAP and KN. The combinations and concentrations of PGRs were shown significant variations for the frequency of callus formation, appearance of callus and the potential of callus differentiation. 2, 4-D (1.0 mg/l) alone and 1.0 mg/l of 2, 4-D with 0.5 mg/l KN have been found highly effective in callusing with quite good in texture and friable in nature i.e. 65.5 % to 75.0 % respectively. After the callus formation the induced callus were sectioned into small pieces and transferred on MS media supplemented with BAP and KN separately or BAP and KN with NAA, IBA and IAA. MS medium containing 1.5 mg/l BAP with 0.5 mg/l NAA was found to be the best medium for maximum *in vitro* response i.e. (80.0 %) shooting and multiple shoots (6.0) with maximum 7.5 cm in length. Best rooting was achieved on full strength MS medium fortified with 1.0 mg/l IBA respectively.

Key words: Indirect organogenesis; *Psoralea corylifolia; In vitro* propagation; endangered; conservation; plant growth regulators.

Introduction

Tissue culture technique is being increasingly exploited for clonal propagation and *in-vitro* conservation of valuable indigenous germplasm that are threatened with extinction [6]. Micropropagation method is specifically applicable to species in which clonal propagation is needed [9]. *Psoralea corylifolia* L. commonly known as 'Babachi' belongs to family Fabaceae (Leguminosae) has been administered as a traditional medicine for years to cure psoriasis, scabies and ringworm infestations. It has been used in Ayurveda as a laxative, aphrodisiac, antihelmintic, diuretic and diaphoresiac in fibril condition. The plant finds use in about many Ayurvedic formulations, which include traditional formulation. It is used in the treatment of febrile diseases, premature ejaculation, impotence, lower back pains, frequent urination, incontinence, bed wetting etc.It is an endangered, herbaceous and medicinally important plant, found in tropical and subtropical region of the world. It grows in the plains of Central and Eastern India. It is reported be cultivated to some extent in the states of Rajasthan and the eastern districts of Punjab [28]. The major active constituents of *Psoralea corylifolia* are psoralen, isopsoralen, neobavaislfoavone, bovachin, bavaislfoavone, bavachro-

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mene, psoralidin, corylifolinin, bavachinin, bavachalcone [12]. Daidzein (4:7 dihydroxuisoflavon) and genistein (4'5'7 trihydroxyisoflavon) are present in natural plants of *Psoralea corylifolia* as well as in *in-vitro* cultures [33]. Almost the plant is used by the Indian traditional system of medicine for the treatment of various skin diseases in human beings. Particularly, the seed of *Psoralea corylifolia* has significant medicinal properties.

The seeds have been specially recommended in the treatment for leucoderma, leprosy, psoriasis and inflammatory diseases of the skin [35]. The roots are useful in the caries of teeth [35]. The one seeded fruits are highly regarded as an aphrodisiac and tonic to the genital organs.The seed of *Psoralea corylifolia* has its commercial price in pharmaceutical market. Due to poor availability, possibilities of facing threats by this species, it is the need of time to increase the population size and ensure the greater biomass availability and conserve the species as well.

The present study describes the indirect organogenesis strategies developed by using tissue culture technology for this species under the influence of different combinations and concentration of certain growth regulators.

Materials and methods

Collection of plant material

Young leaves of *Psoralea corylifolia* from 6 months old plant and were collected from the pot culture maintained in nursery and were used as explants.

Sterilization of explants and preparation of culture

The leaves were thoroughly washed with running tap water to remove all the dust particles adhere with 1 % bavistin for 10 minutes, followed by three rinses with sterile double distilled water. To eliminate other contamination explants were rinsed with 70 % alcohol for 1 minute followed by distilled water washing twice. The explants were then surface sterilized by immersing in a freshly prepared solution of 5.0 % (w/v) NaOCl (Sodium hypochlorite) (Qualigens Fine Chemicals, India) for 5 to 6 minutes under laminar flow. Finally the explants were washed 5 to 6 times with sterile double distilled water for 5 minutes to remove all traces of sterilizing agents used [30].

Callus induction medium

The leaf segments were inoculated on MS media supplemented with auxins either alone in the concentration of (0.5-2.0 mg/l) 2, 4- D or with varied concentration and combinations of cytokinins like (1.5 mg/l) BAP and (0.5 mg/l) KN for callus induction. The cultures were incubated at a temperature of 25±2°C and a photoperiod of 8 hrs light (intensity of 2000 lux) and 16 hrs of dark. Visual observations like callus induction, growth of callus, number of days taken for shoot regeneration and number of shoots regenerated per explants were recorded regularly. A mean of 10 replicates were taken per treatments.

Shoot regeneration medium

For the regeneration of shoots the induced callus were sectioned into small pieces and transferred on MS media supplemented with cytokinins either alone (0.5-1.0 mg/l) BAP and (0.5-1.0 mg/l) KN or different higher concentrations of cytokinins like (0.5-2.0 mg/l) BAP and (0.5-2.0 mg/l) KN with auxins like 0.5 mg/l concentration of NAA, IBA and IAA. The cultures were incubated at a temperature of 25±2°C and a photoperiod of 16 hrs light (intensity of 2000 lux) and 8 hrs of dark.

Shoot multiplication medium

For shoot multiplication the regenerated shoots from callus were separated and transferred on MS media supplemented with different concentration of cytokinins like (0.5-2.0 mg/l) BAP and (0.5-2.0 mg/l) KN either alone or with different combination of auxins like (0.5 mg/l) IBA, NAA and IAA. The growth response of explants were studied at weekly interval. The parameters were taken as the average number of shoots initiated and multiplied, and the length of regenerated and multiplied shoots were recorded.

Rooting of elongated shoots

After proper shoot elongation, the plantlets were properly removed from medium and were transferred into full strength MS medium supple-

mented with different concentrations of auxins like NAA, IAA and IBA for rooting. After one month the complete plantlets were transferred in field for hardening.

Hardening of *in vitro* raised plantlets

The rooted plantlets were washed with 1 % bavistin and transferred to humus rich soil: cocopit filled plastic cups in the ratio 3:1 and were kept under moist chamber in green house for 25days (35-38^{o}C temperature and humidity at 90 % RH). After 25 days the rooted plantlets were transplanted to single net shade house in plastic cups of traditional potting mixture containing soil: compost: coco pit in the ratio 1:1:1 for the secondary hardening.

Results and discussion

Callus induction

Several experiments were conducted to study the effect of different concentration and combination of (0.5-2.0 mg/l) 2, 4-D either alone or with (1.5 mg/l) BAP and (0.5 mg/l) KN on callus induction response by using leaf segment of *Psoralea corylifolia*. On the most media combinations, explants either callused or turned brown in colour. Maximum callus (75.0 %) was induced from the cut ends of explants on medium supplemented with (1.0 mg/l) of 2, 4-D and (0.5 mg/l) KN within 25 days and the callus was greenish, friable and embryogenic in nature (Table 1; Figure. 1 a-c). While as the callus produced in 2, 4-D (1.0 mg/l) alone was greenish brown compact. However reduced callus (40.8 %) was observed when 1.5 mg/l 2, 4-D was supplemented with combination of 0.5 mg/l BAP after 30 days.

The induced calli were subsequently removed and transferred to new different medium combinations for shoot regeneration. Hence in the present study, leaf segments proved to be the best in terms of callus formation. In contrast to the use of other explants, the use of sterile leaf segments to establish the callus cultures would be very convenient for clone elite individuals. This is due to the fact that leaves are easy to obtain and do not require to sacrifice the mother plant. In the present study it is observed that the present investigation revealed the direct contrast to the reports of [21] in *Rauvolfia serpentina*[38]in *Mucuna pruriens* (L.) [23] in *Phyllanthus amarus* and in *Gymnema sylvestris* [2]. Similarly 95 % callus induction were observed from leaf explants on the concentration of (18 µM) 2, 4-D with (2.0 µM) KN[25,26].

Callus regeneration

For the regeneration of shoots, the initiated callus from leaf segments was transferred on fresh

Table 1. Effect of MS media and growth regulators either alone or in combination on callus induction of *Psoralea corylifolia*

No.	MS+auxin/ cytokinin (mg/l)	% Age of callus induction	No. of days required	Morphology of callus	Weight of Callus (mg)	
					Fresh weight	Dry weight
1	0.5 2, 4-D	45.0	30	Dark green	1690	140
2	1.0 2, 4-D	65.5	30	Green brown compact	1850	148
3	2.0 2, 4-D	50.3	35	Dark Brown and compact	1750	145
4	3.0 2, 4-D.	40.2	40	Green	1600	140
5	1.5 2, 4-D + 0.5 BAP	40.8	30	Greenish	1600	141
6	1.0 2, 4-D + 0.5 BAP	33.5	30	Dark green.	1590	139
7	2.0 2, 4-D + 0.5 BAP	30.5	35	Greenish yellow compact	1500	131
8	1.0 2, 4-D + 0.5 KN	75.0	25	Greenish friable.	2000	156
9	1.0 2, 4-D + 0.5 KN	45.4	30	Greenish brown and compact	1700	148
10	2.0 2, 4-D + 0.5 KN	30.5	40	Brownish green soft	1500	112

medium containing different concentration and combination of auxins/cytokinins ranging from 0.5-2.0 mg/l BAP and 0.5-2.0 mg/l KN with 0.5 mg/l of IAA, NAA and IBA. Different types and concentration of auxins and cytokinins were used in the medium and a possible interaction between exogenous and endogenous concentrations of plant growth regulators have a marked effect on the *in vitro* culture responses [35]. Although 100 % cultures showed regeneration in cytokinin/auxin containing medium, but best (80.0 %) shoot regeneration was observed on MS medium supplemented with 1.5 mg/l BAP with 0.5 mg/l NAA of about 6.0±0.2 number of shoots were observed having length of 7.5±0.8 cm. within 20 days. (Table. 2, Figure 1 (d-f).

It was observed that the callus enlarged to adventitious shoot-buds directly from the cut ends of the explants, but few shoot-buds were differentiated from callus derived from leaf segments when the medium supplemented with 2.0 mg/l of KN and 0.5 mg/l of IAA were used. In this combination about 2.6±0.12 average number of shoots having length of 3.1±0.1cm.were observed within 35 days. On the other hand, only 40.0 % of shoot buds were differentiated from callus on the medium supplemented with 1.5 mg/l of KN with 0.5 mg/l of NAA of about 2.0±0.1 number of shoots having length of 3.0±0.1cm.were achieved within 35 days.

Hence, cytokinins particularly 1.5 mg/l BAP with 0.5 mg/l NAA combination acts as trigger and was more effective for shoot regeneration from callus. Similarly the synergetic effect of BAP with NAA were observed in *Glinus lotoides* (L.) [32], *Eclipta alba* L [3], *Casuarina cunninghamiana* Miq [14] and in *Saussurea obvallata1* [8]. However In *Psoralea corylifolia* higher number of shoots (6.15 shoots) were obtained on 14 µM/l BAP with 10.0 µM/l NAA and 10.0 iM/l KN from leaf derived callus [25,26].

Shoot multiplication

In order to get profused rapid shoot multiplication MS medium supplemented with cytokinin and auxin combinations were used. Auxins, like IBA and NAA were added and tested along with varied concentration of BAP and KN. Nevertheless, addition of auxins to the medium did not have a positive effect on multiplication fold and shoot elongation. A prolonged incubation of 6 weeks instead of the usual 5 week culture period results significant increase in shoot formation and elongation.

It was observed that every medium gives response but the cultures showed highest percentage (80.0 %) of shoot formation with an average of 8.0±0.1 adventitious shoots, having length of 10.0±0.8 cm. directly from the regenerated shoots, without any callus formation on medium supplemented with 1.5 mg/l BAP with 0.5 mg/l NAA within 20 days**.** Table 3; Figure 1 (g-h).

Table 2. Effect of MS media and growth regulators either alone or in combination on shoot regeneration from callus of *Psoralea corylifolia*

No.	MS+auxin/ cytokinin (mg/l)	% Age of callus regeneration	No. of days required	Mean no. of shoot produced±SE	Mean shoot length in cm±SE
1	0.5 BAP	40.9	30	1.8±0.5	2.0± 0.0
2	0.5KN	35.5	25	2.0±0.1	2.7±0.14
3	1.0 BAP	68.5	25	4.1±0.0	5.0±0.5
4	1.0 KN	50.2	30	4.0±0.11	4.5±0.11
5	0.5 mg/l BAP+ 0.5 mg/l IBA	55.4	30	4.6±0.1	5.8±0.8
6	0.5 mg/l KN +0.5 mg/l IBA	45.5	35	3.7±0.3	4.5±0.9
7	1.5 mg/l BAP+ 0.5 mg/l NAA	80.0	20	6.0±0.2	7.5±0.8
8	1.5 mg/l KN +0.5 mg/l NAA	40.0	35	2.0±0.1	3.0±0.1
9	2.0 mg/l BAP + 0.5mg/l IAA	65.0	30	4.9±0.7	4.8±0.7
10	2.0 mg/l KN + 0.5 mg/l IAA	42.0	35	2.6±0.1	3.1±0.1

Figure1. Micropropagation of *Psoralea corylifolia*

(a-c) Callus induction from leaf segments on MS+1.5 mg/l 2, 4-D+0.5 KN mg/l after 25 days. (d-f) Shoot regeneration from callus on MS +1.5 mg/l BAP +0.5 mg/l NAA after 20 days. (g-h) Shoot multiplication from regenerated shoots on MS+1.5 mg/l BAP+0.5 mg/l NAA after 20 day. (i-j) Root induction from shoots of on MS +1.0 mg/l 1BA after 20 days and (k-l) Hardening of tissue culture raised plantlets in micro-propagation unit.

Table 3. Effect of MS media and growth regulators either alone or in combination on shoot multiplication from regenerated shoots of *Psoralea corylifolia*

No.	MS+auxin/ cytokinin (mg/l)	% Age of shoot multiplication	No. of days required	Mean no. of shoot produced±SE	Mean shoot length in cm±SE
1	0.5 BAP	40.0	20	3.0±1.2	3.9±0.02
2	0.5 KN	30.5	25	1.0±0.52	2.0±0.3
3	1.0 BAP	50.5	25	4.0±0.6	5.2± 0.01
4	1.0 KN	35.0	30	2.0±0.1	3.0±0.5
5	1.0 BAP+0.5 IBA	55.0	30	4.5±0.6	5.2±0.7
6	1.0 KN+0.5 IBA	45.0	30	3.5±0.6	4.4±0.1
7	1.5 BAP +0.5 NAA	80.0	20	8.0±0.1	10.0±0.8
8	1.5 KN+0.5 NAA	60.0	25	5.0±0.7	6.8±0.2
9	2.0 BAP+0.5 NAA	65.9	25	6.5±0.2	7.5±0.6
10	2.0 KN+0.5 NAA	50.5	30	4.3±0.1	5.8±0.9

However, only few shoots has been observed on KN in the concentration of (1.5-2.0 mg/l) with IBA and NAA. An average of 50.0- 60.0 % of shoot multiplication was observed which is of 4.3±0.1 in number having length of 5.8±0.9 cm over a period of 30 days. Therefore, from all concentrations 1.5 mg/l BAP with 0.5 mg/l NAA produced the most desirable results both in terms of multiplication fold and cluster of perfective elongated shoots. The obtained results are parallel with the earlier reports on *Psoralea corylifolia* for highest rate of shoot multiplication[13,19]. In contrast to the synergistic effect of BAP in combination with NAA has been also previously reported in *chrysanthemum* [1],*Alstroemeria* cv. "Fuego,[16], and also in *Chrysanthemum morifolium* [10,11,7,27,31,36,18,20].

Rooting of *in vitro* regenerated shoots

The induction of roots has been observed in every medium tried. Following the protocol of [19] elongated and well developed regenerated shoots via callus were aseptically excised and implanted on MS medium supplemented with root inducing growth regulators at the concentration of (0.5 to 1.5 mg/l) IAA, (0.5 to 1.5 mg/l) NAA and (0.5 to 2.0 mg/l) IBA were tested. Maximum (70.5 %) root induction was achieved directly from the base of the shoots on medium supplemented with 1.0 mg/l IBA of average length 6.0±0.9 cm.within 20 days. Table 4; Figure 1 (i-j). On the contrary, by increasing the concentration of IBA results decrease in root formation,as observed only (55 %) percentage of root induction was observed on higher (2.0 mg/l) concentrations of IBA**.** (Table 4). Similar results were reported in *Psoralea corylifolia* by [4,5,13,19,22,24,25,26]. Superiority of IBA for root induction has been reported earlier in many other plant species such as in *Glinus lotoides* (L.)[32], *Eclopta alba* L [3],*Chrysanthemum* [10,11,15,6, 17,29,]

Identification of suitable hardening medium for better establishment

After one month of root development, 15 plantlets were transferred to green house for primary and secondary hardening. The survival was recorded as 100 % in the transplantation. After one month the fully matured plantlets were transferred to open nursery for exposure to direct sunlight, showed 100% survival with flowering within next 20 days Figure 1(k-l). Hence the *in-vitro* indirect organogenesis and elite plantlet production protocol establishment of a medicinal plant species, *Psoralea corylifolia* from leaf explants by employing plant tissue culture technique is more useful to enhance the population and the biomass of this endangered species.

Callus growth measurements

The growth of the shoot callus cultures was

Table 4. Effect of MS media and growth regulators on root induction of *Psoralea corylifolia*

No.	Media composition (mg/l)	% age of root induction	No. of days required	Mean root length in cm ± SE
1	MS +0.5 IAA	45.0	30	3.5±0.7
2	MS +0.5 IBA	50.0	25	4.0±0.3
3	MS +0.5 NAA	40.0	20	3.0±0.3
4	MS +1.0 IAA	40.5	20	3.0±0.9
5	MS +1.0 IBA	70.5	20	6.0±0.9
6	MS +1.0 NAA	45.5	25	3.7±0.6
7	MS +1.5 IAA	55.5	25	4.5±0.2
8	MS +1.5 IBA	60.5	25	5.0±0.9
9	MS +1.5 NAA	50.0	30	3.2±0.2
10	MS +2.0 IBA	55.0	25	4.4±0.8

measured in terms of fresh weights (FW) as well as dry weight (DW). The explants were placed between the folds of blotting paper to remove excess moisture and the fresh weight was then determined. Dry weight was measured after drying the fresh materials in an oven at 60 ± 1°C for 48 hrs.

Conclusion

In the present study, a fruitful indirect organogenesis protocol was set up for *Psoralea corylifolia* through callus formation, shoot regeneration and successful regeneration of new plantlets. However according to the literature in the present study *in vitro* raised plantlets of *Psoralea corylifolia* through indirect organogenesis was observed on MS media supplemented with lowest concentration of plant growth regulators as well as within minimum time period. The result of this study shows that tissue culture techniques can play an important role in clonal propagation of elite genotypes of *Psoralea corylifolia* which has diverse medicinal applications and eventually due to over exploitation and irregular concern this plant is facing local extinction.

In conclusion we demonstrate that protocol developed could be used for conservation of elite germplasm and true to type mass propagation of this herb of immense pharmaceutical relevance. This is highly advantageous for the production of uniform source of *Psoralea corylifolia* plants for a range of further biotechnological applications and will also help in the production of improved plants.

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