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In vitro propagation of *Psoralea corylifolia* L. -An important *Endangered* medicinal plant

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Abstract

Psoralea corylifolia L. belongs to the family Fabaceae. Its natural population has declined very fast due to indiscriminate and illegal collections and destruction of its habitats and as a result it is included in the endangered list of plants. It's *in vitro* protocol techniques are currently unavailable to help growers to meet the demand of the plant for cultivation and pharmaceutical industry.

The present study deals with rapid and efficient protocol development for *in vitro* propagation of Shoot induction on Murashige and Skoog medium supplemented with various auxins and cytokinins individually and in various combinations has been achieved by using axillary and apical meristems.

MS medium fortified with 0.5- 2.0 mg/l BAP and 0.2mg/l NAA was found to be effective individually.

The medium with 0.5mg/l BAP + 0.2 mg/l NAA responded better as compared to other combinations. 3-6 shoots having 2-3cm length has been initiated from axillary meristem were excised and further used for shoot multiplication on MS fortified with high concentration of growth hormones to produce shoots.

Key words: *Psoralea corylifolia* medicinal plant, conservation, Tissue culture, Growth regulators. Endangered medicinal plant.

Plants are being used as source of medicine since ages. Many medicinal plants are nature's gift to human beings to make disease free healthy life. More than 80% of the world population are in poor and less developed countries depends on traditional plant based medicines for their primary health care needs. 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[5]. The use of different parts of several medicinal parts to cure specific diseases has been in vogue from ancient times.

Psoralea corylifolia L. (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[4].

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*[2,3].

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[1] Many Indian pharmaceutical industries have used *P. corylifolia* as a raw material in the production of medicines and Ayurvedic skin care soap[1]. 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.

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. Sterilization of explants Auxillary Apical meristems that are of 1mm 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 40 min, and treated with liquid detergent for 50 min, and rinsed three times with distilled water. Then explants were treated with an antifungal agent (Bavistin) for 3 hours and again rinsed five 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 5 minutes under aseptic conditions. After this these explants were then thoroughly washed 2-4 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 1.5 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: (0.5-2.0 mg/l), 1-Naphthaleneacetic acid (NAA:

0.1- 2.0 mg/l) formulation for shoot proliferation and multiplication. All cultures were incubated under 16h photoperiod with light intensity of 55µmolm–2s–2 provided by cool white fluorescent lamps (Phillips, India) at 25 ± 2°C. All the cultures were transferred to fresh medium after 2 week duration. The frequency and number of shoots formed were evaluated after 3 weeks of inoculation. Morphological changes were recorded on the basis of visual observations at 3-week intervals.

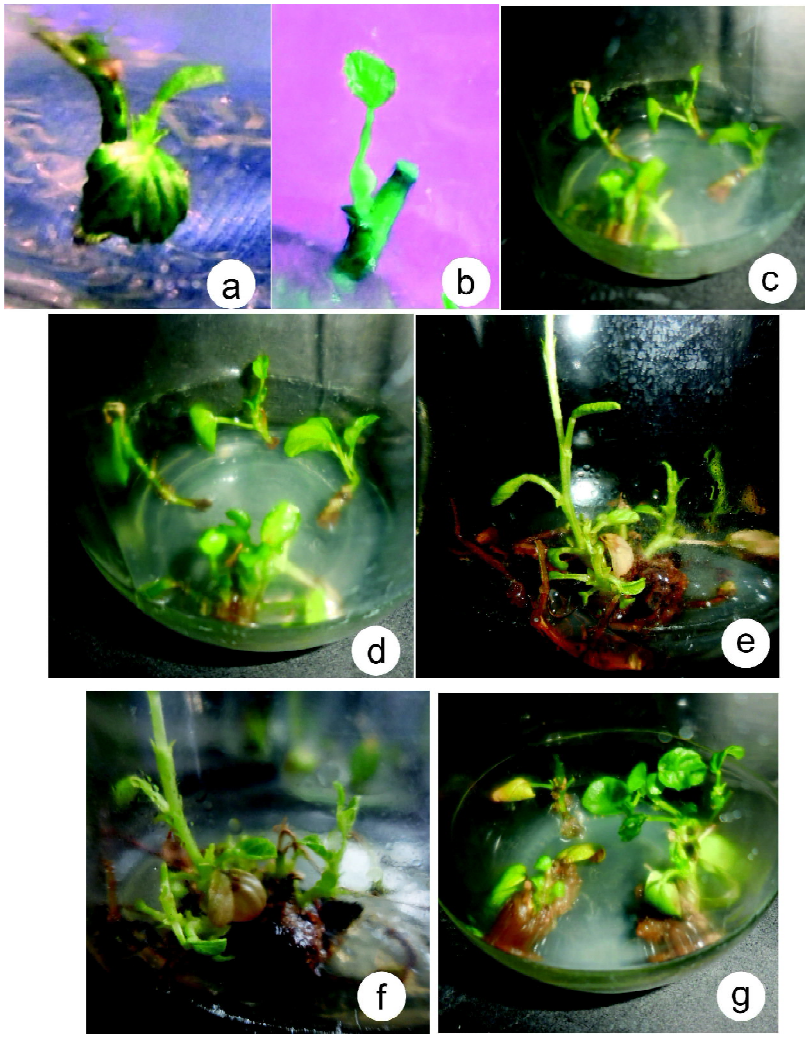
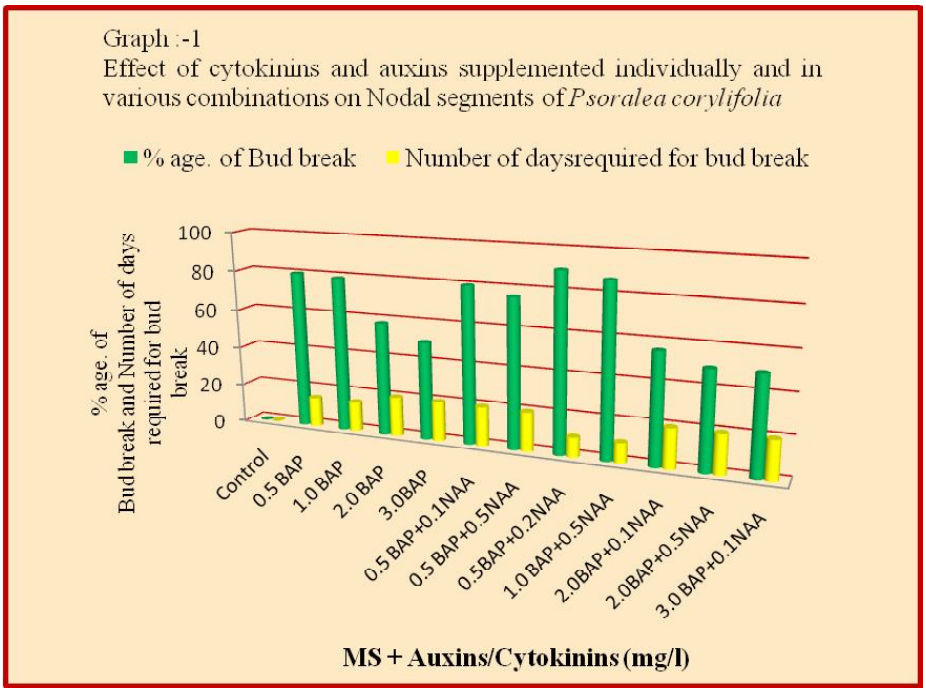


Fig. 1. (a,b) shoot initiation in 0.5 BAP, (c,d,e) shoots in 0.5 BAP + 0.1 NAA, 5- shoot induction after 21 days in 0.5BAP + 0.2NAA and (f,g) shoot multiplications

Table-1. Effect of cytokinins and auxins supplemented individually and in various combinations on Nodal segments of *Psoralea corylifolia*

MS+Auxins/ Cytokinins(mg/l)	%age. of Bud break	Number of days required for bud break	Mean No of Shoots produced ± SE	Mean Shoot Length in (cm) ± SE
Control	0	0	0	0
0.5 BAP	80	21	1.55±0.01	1.38±0.08
1.0 BAP	79	15	1.78±0.01	1.10±0.35
2.0 BAP	58	20	1.57±0.13	1.80±0.42
3.0 BAP	50	20	1.30±0.05	1.58±0.13
0.5 BAP+0.1NAA	80	20	2.00±0.46	1.05±0.31
0.5 BAP+0.5NAA	76	20	1.21±0.02	1.78±0.01
0.5 BAP+0.2NAA	95	21	6.04±0.15	2.27±0.10
1.0 BAP+0.5NAA	87	10	2.27±0.10	1.80±0.11
2.0 BAP+0.1NAA	56	20	1.80±0.42	1.50±. 0.09
2.0 BAP+0.5NAA	50	20	1.01±0.04	1.70±0.08
3.0 BAP+0.1NAA	**50**	**20**	**1.27±0.10**	**1.30±0.19**

Graph :-1
Effect of cytokinins and auxins supplemented individually and in various combinations on Nodal segments of *Psoralea corylifolia*



After 2-3 weeks from culture initiation, shoots appeared and increased on subsequent sub-culturing. 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 alone facilitated the shoot differentiation Among the various combinations of BAP, and NAA, highest Shoot regeneration (95%) results were obtained on MS medium containing BAP (0.5 mg/l) with NAA(0.2mg/l) generating shoots (6.12 shoots) When BAP concentration was increased above 15 mg/l, the rate of shoot multiplication was reduced. Increasing concentrations of NAA (>15 mg/l) decreased shoot number and length. 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.

The micro propagation protocol reported here was characterized with a rapid proliferation of shoots. 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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