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Effect Of Different Growth Hormones On In Vitro Response Of A Leguminous Medicinal Herb

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Abstract: Medicinal plants are the local heritage with global importance and world is endowed with a rich wealth of medicinal plants. *Psorelea corylifolia L.* is an important medicinal plant belonging to Leguminosae family, distributed in tropical and subtropical regions. It is used as medicine to treat many diseases. Pharmaceutical companies largely depend on natural plants to procure materials causing depletion of this important medicinal herb. Hence, the present study has been taken up with two aims: to establish a suitable protocol for rapid proliferation; Invitro regeneration of through shoot bud formation from hypocotyl and leaf explants. MS medium supplemented with various combinations and concentrations of plant growth regulators was used. MS media with Auxin NAA 0.5 mg/lt and BAP 5mg/lt or 10mg/lt showed highest frequency of shoot. Regenerated shoots were rooted in MS media with 5mg/l and 10mg/l IBA. Shoot budding occurred directly from the explants as well as from callus. The present study is a maiden attempt to assess the regenerative capacity of the explants under different hormonal conditions. Regeneration was much higher from hypocotyl than from leaf.
Keywords: Different Growth Hormones, In Vitro Response,A Leguminous Medicinal Herb.

Introduction :

Medicinal plants are the local heritage with global importance and world is endowed with a rich wealth of medicinal plants. Plants have a great potential to be effective against the growth of pathogenic bacteria. *Psorelea corylifolia* L. Commonly known as 'Babchi' is an important endangered medicinal herb belonging to leguminosae family, distributed in the tropical and subtropical regions[1]. It is used as aphrodisiac,laxative, diuretic, anthelmintic and diaphoretic in febrile conditions. Pharmaceutical companies largely depend upon materials procured from naturally occurring stands, causing rapid depletion of this important source of medicinal herbs. Propagation of Psorelea through seed is restricted due to its reduced span of viability & low germination rate. Wild seedlings of this plant exhibit high mortality. Its natural population has declined very fast due to indiscriminate and illegal collections and destructions of its habitat[2].

Hence, it has become imperative to establish a suitable protocol to generate enough materials to ensure its supply for pharmaceutical industries without further depopulating this species. Plant tissue culture techniques offer a viable tool for mass multiplication and conservation of rare & endangered medicinal plants for meeting the pharmaceutical needs[3]. Limited tissue culture work has been done on Psorelea corylifolia[4,5]. The present study explains an efficient in vitro method for rapid mass propagation of P. Corylifolia from various explants using different combination & concentrations of auxins & cytokinin.

Material and Method:

Seeds of *Psorelea corylifolia*, obtained from Local medicinal plant agency, were surface sterilized with 0.1% mercuric chloride (W/V) for 5 min and then rinsed in sterile distilled water 4-5 times. The seeds were germinated on sterile moist filter paper in Petri plates/sterile moist cotton in flasks at 22-55^{0}c in dark.

Hypocotyl and leaf explants were excised from 3 to 4 days old seedlings and placed aseptically on solidified MS medium containing 3% sucrose and 0.8% agar. The pH of the media was adjusted to 5.6-5.8 with 0.1N sodium hydroxide and/or 0.1 N hydrochloric acid prior to adding the agar. The media were supplemented with filter sterilized auxins 1-Naphthaleneacetic acid (NAA), Indolebutyric acid (IBA), 2, 4 Dichlorophenoxyacetic acid (2, 4-D) and Cytokinin 6-Benzylaminopurine (BAP) both individually and in combination at a concentration of 0.1 – 10mg/litre. Cultures were maintained under white fluorescent light with light dark cycles of 16 hr/8 hr at 25 ± 2^{0}C. After shoot induction they were subculture onto MS medium fortified with various concentrations of IBA for root induction. The rooted shoots were removed from the culture medium and transferred to plastic cups containing a mixture of garden soil, and sand (2: 1). Potted seedlings were grown under laboratory conditions of regulated humidity and temperature for two weeks, irrigated with knops' soln. once every three days. The plants were kept under shade for four weeks and then placed under full sunlight. Data collected after 35-40 days of culture for shoot and root response. Only data which showed some advantageous effect are included in the tables. All the experiments were repeated three times with19 explants per treatment. The statistical significance was calculated by using ANOVA and significance at 5% level.

Result and Discussion :

The effect of various conc. of the present study Auxin alone and in combination with Cytokinin on shoot multiplication and complete plantlet regeneration was evaluated. The highest shoot regeneration frequency (85 % to 90 %) was observed in the medium augmented with high conc. of BAP (10 mg / l) alone, while low conc. of 2, 4-D (.1 mg / l) showed moderate frequency (70 %) of shooting. :Moderate to high conc. (2.5 mg / l – 5 mg / l) of NAA also showed 70 % frequency of shoot induction. 2, 4-D at different concs., was effective for callus but not found suitable for shooting at high conc. when used alone & in combination with BAP. Suppressive effect of 2, 4-D for shoot induction at higher concentration is also reported earlier by several workers[6,7]. BAP alone when used in different conc. showed high frequency of shoot induction in all the 3 explants used. Similar effect of BAP alone on *Psorelea corylifolia* has been found in previous studies[8-10].

In combined treatment of Auxin &Cytokinin (BAP), high conc. (5 mg/l and 10 mg / l) of BAP with low to moderate conc. (0.5-2.5 mg / l) NAA exhibited high rate of shoot regeneration i.e. (90 %). The synergistic effect of auxin and cytokinin has been demonstrated in several medicinal plants viz. *Rawvolfia tatraphyla*[11], *Rotulla aquatica*[12], and *Santolina canescense*[13]. The present study also supported the shoot induction capacity of combined treatment of high conc. of BAP with low conc. of NAA. When BAP conc. was increased above (10 mg / l), frequency of shoot regeneration sharply reduced, similar result has been observed in many medicinal plants such as *Malus sylvestris*[14], *Piper* sps. [15], *Plumbago zeylanica*[16].

All the regenerated shoots were transferred to various conc. of IBA, IAA, NAA alone and in combination with BAP & KN. Moderate to high conc. (2.5 mg / l – 10mg / l) IBA alone proved to be most effective for rooting. Out of the 3 auxins(IAA, NAA, IBA) tested for rhiogenesis, IBA was found to be superior than others. Effectiveness of IBA on Rhizogenesis has been reported in past in *sesbania aculeata*[17], *Cephaelis ipecacuanha*[18] and some medicinal plants like *Aloe polyphylla*[19] and *Tylophora Indica*[20] etc. Rooted plantlets were transferred to pots containing 2:1 garden soil and sand irrigated with knop's soln. covered with transparent polythene bags; subsequently they were transferred to larger pots and gradually acclimated to outdoor conditions.

The present study has been taken up to establish a suitable protocol for rapid proliferation and in vitro regeneration of *Psorelea corylifolia* L.and deals with complete regeneration of *Psorelea corylifolia*. The study indicates that shoot regeneration is the result of interaction between Auxin &Cytokinin in various concentrations and combinations in the medium, while rhizogenesis in regenerated shoot can be obtained by Auxin alone in the medium. Similar effect was also observed by some workers in recent studies[21].The present study is a maiden attempt to assess the regenerative capacity of the explants under different hormonal conditions. The method used in the study is reproducible and can be applied for germplasm conservation & large scale regeneration of important medicinal plants.

Table- 1: Response of different explants to various growth regulators on shoot formation

Growth regulators (mg/l)	Type of explants	%explants producing shoots	No. of Explants producing shoot (mean± SE)
1 NAA	H	70%	13.3± 0.25
2.5 NAA	L	38%	6.6± 0.35
	H	68%	12.6± 0.30
5 NAA	H	70%	12.66± 0.22
0.1 (2,4-D)	L	22%	3.6± 0.35
	H	70%	14± 0.16
0.5 (2,4-D)	H	14%	2.6± 0.36
5 BAP	L	64%	11.6± 0.30
10 BAP	L	84%	16.3± 0.22
	H	94%	18± 1.12
5BAP+10NAA	L	38%	6.66± 0.16
5BAP+1NAA	L	74%	14± 0.17
	H	90%	17± 0.38
10BAP+1NAA	L	64%	12± 0.32
	H	70%	13.33± 0.22
10BAP+2.5NAA	H	92%	17.66± 0.30
0.1 2,4-D+5BAP	H	82%	15.66± 0.30

L=Leaf, H=Hypocotyl.

Table II: Micro shoots producing roots

Growth regulators	% Micro shoots producing roots (mean± SE)	No. of Micro shoots producing roots (mean± SE)
0.5 IBA	20%	3.6±0.350
2.5 IBA	70%	13.3±0.252
5 IBA	85%	16.3±2.5
10 IBA	95%	18±0.519
0.5 BAP+10 IBA	60%	11.3±0.246
0.5KN+10IBA	65%	12.3±0.309

Values are mean± Standard Error (SE). Mean with standard error of three repeated experiment.

- Shoot bud induction in MS Media with 1mg /l NAA and5 mg /l BAP.
- Multiple shoot in 2.5mg /l NAA and 10 mg/l BAP
- MS media with 5mg /l BAP and 10 mg/l I BA
- Plant let in pot for hardening irrigated with Knop's solution.

MS Media with 1 mg/l NAA & 5 mg/l BAP

MS Media with 5 mg/l BAP & 10 mg/l IBA

MS Media with 2.5 mg/l NAA & 10 mg/l BAP

MS Media with 5 mg/l BAP & 10 mg/l IBA

Potted Plant let

Psorelea Plant

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