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In-vitro callus induction and shoot regeneration in *Ipomoea obscura* (L.): potent Indian medicinal plant

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Abstract: Callus of the leaves, node and bud of *Ipomoea obscura* (L) was initiated on MS basal media supplemented with various combinations of auxins 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthalene acetic acid with cytokines kinetin and benzyl amino-purin (BAP). Callus initiation was observed in all media but with varied mass. Highest percentage of callus response was obtained in combination of 2,4-D (0.8 mg/L) with kinetin (0.8mg/L); NAA (0.2mg/L) with BAP(0.2 mg/L); NAA (0.8 mg/L) with kinetin (0.8 mg/L) for explants leaves, node and bud, respectively. The callus was white in 2,4-D- and green in NAA- supplemented MS media. An efficient micropropogation protocol was developed for medicinal plant *Ipomoea obscura* (L.) by *in-vitro* culture of nodal part of mature plant. The MS media supplemented with 0.8 mg/L NAA with 0.8 mg/L kinetin induced three shoots per node in an average and was best for axillary bud proliferation.

Keywords: Ipomoea obscura, tissue culture, medicinal plant

Introduction

In vitro propagation is an important tool for rapid multiplication of medicinal plants (Atal & Kapur 1982 a, b) as well as for the extraction of active ingredients. *Ipomoea obscura* (L) commonly known as Laksmana in ayurveda belongs to the family Convolvulaceae. It is small clambering vine, leaves small, cordate, apex acuminate, corolla composed of fully fused petals, white colored, annual plant. Prefers rocky slopes, fences or low ground cover as a substrate (Singh *et al*., 2005). The leaves collected from wild are eaten as a cooked vegetable in Kenya and added to soup in Nigeria. Ayurveda has identified many medicinal properties of this plant and it is effectively used against dysentery (Christophe *et al*., 2002; Eckart Esich, 2008). Choosy leaves with alcohol are applied to open sores and pustules. A pest of leaves is applied on ulcers, hemorrhoids and swellings. Seed and fruit are used as cleaning agents, to improve difficult breathing, relive pain and to improve vision. It is being used as an antioxidant (Shrinivasan *et al*., 2007). It also has ornamental value as a climber with attractive flower (Shahina, 1994).

The plants of this family are extensively investigated as a newer source of natural antioxidants and for other bioactive compounds of human benefits (Lakshmi *et al*., 2000). Due to its seasonal availability and endemic distribution the present investigation was focused on to obtain callus from leaves, node and bud. The *in-vitro* multiplication may benefit as the perennial source for the isolation of bioactive compounds.

Material and methods

Plant material

Healthy plant of *Ipomoea obuscra* (L.) was collected from Lourd Mata Mandir, Seminary hills, Nagpur, India. The different parts of plants like leaves, node and bud were washed with tap water and then with detergent (Teepol) for 15 min followed by washing with distilled water.

Preparation of explants

Leaf as explants: The leaf explants were cut into small pieces and washed with running water. Then, the explants were surface-sterilized with 0.1% (w/v) mercuric chloride for 2-3 min, followed by 70% ethyl alcohol 2-3 min, washed 3-4 times with sterile double-distilled water and inoculated on agar-solidified MS (Murashige & Skoog) medium supplemented with different concentrations of 2,4-D, kinetin and BAP, either alone or in combination, with 3% (w/v) sucrose. The pH of the medium is adjusted to 5.8 before sterilization. Cultures were maintained at 27±1°C photoperiod.

Node as explants: Nodal region was surface sterilized (similar to leaves) with 0.1% mercuric chloride and 70% ethyl alcohol and cultured on agar solidified MS medium supplemented with different various combinations and concentrations of Auxins 2,4 D (0.1-0.8 mg/L), NAA (0.2,0.4 and 0.8 mg/L) and cytokines kinetin (0.1-0.8 mg/L), or BAP (0.2 mg/L) with 3% (w/v) sucrose. The pH of the medium was adjusted to 5.8 before sterilization. Cultures were maintained at 27±1°C photoperiod.

Bud explants: Bud explants were surface-sterilized with 0.1% (w/v) mercuric chloride for 2-3 min, followed by 70% ethyl alcohol 2-3 min, then washed 3-4 times with sterile double-distilled water and inoculated on agar-solidified MS medium supplemented with different with various combinations and concentrations of auxins 2,4 D (0.1-0.8 mg/L), NAA (0.2,0.4 and 0.8 mg/L) and cytokines kinetin (0.1-0.8 mg/L), or BAP (0.2 mg/L) with 3% (w/v) sucrose. The pH of the medium was adjusted to 5.8 before sterilization. Cultures were maintained at 27±1°C photoperiod.

The experiment was terminated after an interval of 30 days. In another set of experiments where the shoot regeneration capacity was determined, for this node explants was inoculated in the tubes containing MS medium supplemented with kinetin and NAA in concentrations. At least 10 tubes were inoculated and incubated under optimal condition as defined above. After 30 days, the experiment was terminated and shoot generation capacity, its length and morphology were recorded.

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Shoot culture

Basal medium used for initial set of experiment for shoot proliferation consisted of MS salt 3% (w/v) sucrose, and 0.9% (w/v) agar. The pH of medium was adjusted to 5.7. The basal medium was supplemented with various combinations of auxins (NAA, 2,4-D) with cytokines (Kinetin, BAP) at different concentration. The cultures were incubated at 27 ± 1°C under 16 hr photoperiod with cool white fluorescent light.

Results and discussion

Callus Induction

All the combinations of NAA, 2, 4-D with kinetin and BAP produced callus (Table 1&2). Optimum concentration of auxins and cytokines which initiated callus with high percentage of response was used for further study. Callus induction was different in different explant with different hormone concentrations. The highest response of callus formation in leaves (93.3±2.54 %) was observed in MS medium supplemented with 0.8mg/L 2,4-D plus 0.8mg/L kinetin which resulted in white soft callus. The combination of NAA (96.6±0.96% 0.2mg/L) with 0.2 mg/L of BAP gave green hard callus. Callus induction was observed after 7 days of culturing of leaf samples (Table 1, 2; Plate 1).

The highest response in node explant (83.3±2.5%) was observed on MS medium supplemented with 0.4mg/L 2,4-D with 0.4mg/L kinetin which resulted in white soft callus; while, 86.6±2.54% 0.2mg/L of NAA with 0.2mg/L BAP resulted in green hard callus. Callus induction was observed after 6 days in case of node (Table 1, 2; Plate1). These results are in support of earlier investigations carried out for callus induction in *pterocapus santhalinus L*. using node as explant (Rajeshwari *et al.,* 2008).

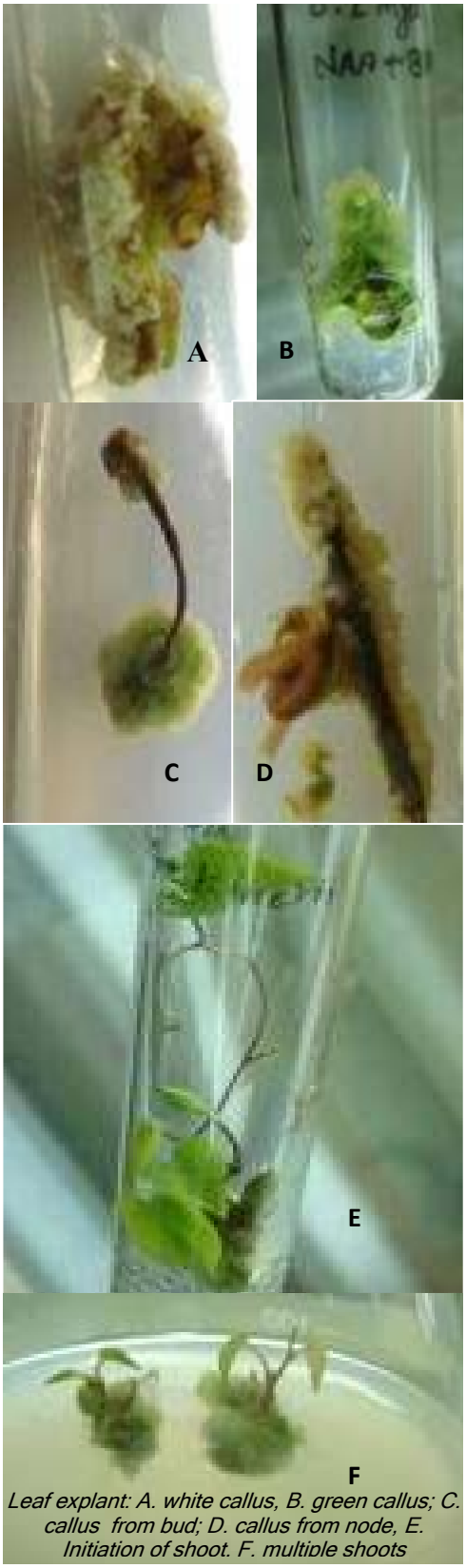
The highest response of callusing was observed in bud explant on MS medium supplemented with 0.2mg/L of 2, 4-D plus 0.2 mg/L of kinetin which resulted in white callus (83.33±0.9). While, 0.8mg/L of NAA with 0.8mg/l kinetin resulted in green callus (96.6±0.96%). Callus induction was reported in 6 days of culturing in medium with 2, 4-D plus Kinetin and 4 days in NAA plus Kinetin (Table 1, 2; Plate1). Growth of callus increased significantly and this covers the entire surface of the explant. In general, it was observed that NAA was the best source of auxin for callus induction along with kinetin (80- 100%) or with BAP (90-96%); then 2, 4- D with kinetin (40-90%) or with BAP (55-80%) (Table 1, 2). The callus produced by 2,4-D was white in contrast to dark green in NAA. MS medium is frequently used for micropropogation of large number of plants (Feyissa *et al.,* 2005). It was reported that *T. bellerica* cultures grew better on MS medium in comparison to all other medium (Rathore *et al.,* 2008). The medium for in -vitro multiplication of *Drosera* plants was MS medium. MS was reported superior medium for micropropogation of *Coptis teeta* (Pramod Tandan, 2007).

Further studies were carried out for shoot regeneration capacity of the node. Shoot were initiated from the node explants by showing both direct and indirect organogenesis. The best result of shooting (7.4 cm; 4 shoots per treatment) was observed in MS medium supplemented with the combination of Kinetin (0.8 mg/l) and NAA (0.8 mg/l) after 17-19 days (Table 3; Plate1).

Conclusion

Depletion of wild population can be prevented through such *in-vitro* cultivation for further commercial exploitation. Amongst all the explants tried for callus, the leaf was found to be the most suitable explant for callusing. NAA and BAP were found to be the most appropriate hormone concentration for callusing. Thus, the present investigation provides optimum parameters for callus and shoots induction. The multiple shooting of *Ipomoea obscura* (L). was also established for single

Plate1. Different stages of in-vitro regenerated I. obscura L.



Leaf explant: A. white callus, B. green callus; C. callus from bud; D. callus from node, E. Initiation of shoot. F. multiple shoots

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Table 1. Effect of different concentration of 2, 4- D with kinetin and BAP on callus induction

Auxins (mg/L)	Cytokines (mg/L)		% of callus response			Nature of callus
2,4D	Kinetin	BAP	Leaf Explants	Node Explants	Bud Explants	
0.1	0.1	--	90±1.66	70±1.6	46.6±2.5	White, Soft
0.2	0.2	--	86.6±2.5	83.3±2.5	83.33±0.9	White, Soft
0.4	0.4	--	85±1.6	80.3±1.5	NR	White, Soft
0.8	0.8	--	93.3±2.54	73±1.07	NR	White, Soft
0.1	--	0.2	56.6±0.96	79.6±1.5	NR	White, Soft

Media - MS +3% sucrose, NR = No Response, Mean value of three readings.

Table 2. Effect of concentration of NAA with kinetin and BAP on callus induction

Auxin (mg/L)	Cytokines (mg/L)		% of callus response			Nature of callus
NAA	Kinetin	BAP	Leaf Explants	Node Explants	Bud Explants	
0.2	--	0.2	96.6±0.96	86.6±2.54	91.6±0.96	Green, Hard
0.4	0.4	--	80±1.66	78.3±0.96	86.67±0.96	Green, Hard
0.8	0.8	--	93.3±0.96	NR	96.6±0.96	Green, Hard

Media: MS + 3%sucrose; NR= No response; Mean value of three readings

nodal region on MS medium supplemented with NAA (0.8 mg/L) and Kinetin (0.8 mg/L). Thus this study provides a standard protocol to initiate multiple shoot culture, optimization of media content and hormonal concentration that may provide desired source of pharmacologically active plant constituents through callus culture.

Table 3. Effect of different concentration of hormone(s) NAA (0.8 mg/L) and Kinetin (0.8 mg/L) on shoot regeneration and height attained from node explant of Ipomoea obscura (L.).

No of test tubes Inoculated	No of shoot per treatment	Shoot length in cm.	Shoot morphology
1	2	3.6	Thin Short
2	2	2.5	Thin Short
3	3	3.1	Thin Short
4	2	5.2	Green and long
5	4	3.8	Thin Short
6	2	7.4	Green and long
7	1	3.2	Thin Short
8	NR	NR	High callus induction
9	NR	NR	High callus induction
10	NR	NR	High callus induction

NR = No response

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