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Full Length Research Paper

Induction of callogenesis in *Ipomoea obscura* (L.) Ker-Gawl, a little known medicinal plant

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The present study was undertaken to explore the potentiality of *in vitro* micropropagation (by callus induction) of *Ipomoea obscura* (Convolvulaceae), a perennial herb. This medicinal plant grows widely throughout Indian subcontinent. Axenically grown cotyledonary leaves regenerated into profuse calli, in various combinations of naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and 2,4-dichlorophenoxyacetic acid (2,4 D) with benzylaminopurine (BAP). Microroots emerged from these calli when subjected to various combinations of NAA and BAP. Maximum number of root meristems and microroots were formed in 1.25 mg/L NAA with the combination of 0.5 mg/L BAP (1:4 v/v). Leaf, nodal and internodal segments as explants were cultured on MS media containing 2,4 D at 0.5, 1.25, 2.50, 5.0 and 10.0 mg/L, for callus induction. Only leaf tips initiated callogenesis with highest response at 10 mg/l 2,4 D and lowest with 0.5 mg/l 2.4 D. This is the first report of *in vitro* response of *I. obscura*.

Key words: Callogenesis, *Ipomoea obscura*, *in vitro* culture.

INTRODUCTION

Ipomoea obscura (L.) Ker-Gawl is a perennial herb and a medicinal plant growing widely throughout Indian subcontinent. Leaves of the plant find valuable application in aphthous affection (Kirtikar and Basu, 1999). In Indonesia, paste of the leaves is applied on sores, ulcers, hemorrhoids and swellings (Wiart, 2006). The plant grows vigorously in rainy season, mainly from June to August. The ideal flowering time of the plant is from October to January (Pankaj Oudhia, 2003). Field studies revealed dwindling population of the plant, which may be due to high and prolonged seed dormancy. This paper proposed an effective conservation strategy for this promising medicinal plant, though simple but effective callus induction. Such calli would be an effective source for future micropropagation studies, specially its phytochemical profile. Very recent report suggested that separation of methanolic seed extract of *I. obscura* yielded five indole alkaloids, three of them (ipobscurines B, C, D) being new natural products of unique structural type characterized as serotonin, hydroxycinnamic acid amide type conjugates with a second phenylpropanoid moiety forming an ether with the 5-OH position of the indole nucleus and neoligans (Jenett-Siems et al., 2003). Recent reports revealed that, *I. obscura* (L.) enhances the functions of immunological effector cells, inhibits proinflammatory cytokines and nitric oxide production by Lipopolysaccharide (LPS) induced macrophages (Hamsa and Kuttan, 2008). Another finding suggested the augmentation of cellular immune response by *I. obscura* and ipobscurine alkaloid, which attenuates tumor growth in mice (Hamsa and Kuttan, 2011). This investigation was undertaken to find out suitable explants for callus induction and subsequent micropopagation of *I. obscura.*

MATERIALS AND METHODS

Mature seeds of *I. obscura* (L) Ker-Gawl were collected in February from natural plants growing in Hooghly District, West Bengal and were sun dried. Then, they were stored in desiccators. Two types of explants were used: one was *in vitro* generated leaf explant and the other was *in vivo* generated leaf explants. All these explants were cut into small pieces (1.0 and 1.5 mm) then, they were treated with 0.1% $HgCl_2$ solution containing 2 to 3 drops of Tween 20 for 5 to 7

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Table 1. Production of *I. obscura* (L.) callus from leaf explants in different concentrations of (1) indole-3-acetic acid (IAA) and benzylaminopurine (BAP) (2) indole-3-butyric acid (IBA) and benzylaminopurine (BAP) (3) naphthaleneacetic acid (NAA) and benzylaminopurine (BAP) (4) 2,4-dichlorophenoxyacetic acid (2,4-D) and benzylaminopurine (BAP).

Hormone combination (mg/l)		Leaf explants producing callus (%)*	Dry weight of callus in mg**
IAA	**BAP**		
0.5	10.0	75.26 ± 1.05	2.04 ± 0.026
1.25	10.0	74 ± 1.37	2.17 ± 0.049
2.5	5.0	74.23 ± 1.87	2.29 ± 1.12
5.0	10.0	98.33 ± 1.52	2.07 ± 0.070
10.0	10.0	99 ± 1.0	2.04 ± 0.096
IBA	**BAP**		
0.5	5.0	0.00	0.00
1.25	10.0	25.03 ± 0.70	1.23 ± 0.015
2.5	10.0	24.96 ± 0.58	1.29 ± 0.015
5.0	10.0	74.3 ± 0.75	2.17 ± 0.070
10.0	10.0	98.9 ± 0.95	2.29 ± 0.096
NAA	**BAP**		
0.5	1.25	76.5 ± 1.41	3.24 ± 0.086
1.25	5.0	75.4 ± 0.20	3.54 ± 0.157
2.5	10.0	100.03 ± 0.26	3.51 ± 0.050
5.0	10.0	98.7 ± 0.80	3.80 ± 0.117
10.0	10.0	100.3 ± 0.26	3.90 ± 0.026
2,4D	**BAP**		
0.5	10.0	100.33 ± 0.20	4.45 ± 0.179
1.25	10.0	100.33 ± 0.15	4.47 ± 0.104
2.5	10.0	100.23 ± 0.32	4.45 ± 0.177
5.0	10.0	100.4 ± 0.10	5.44 ± 0.160
10.0	10.0	100.4 ± 0.26	5.45 ± 0.267

*Average response of three replicates of callus and data (± SD) were recorded up to five weeks of culture. **Average response of three replicates of dry weight of callus and data (± SD) were recorded up to five weeks of culture.

min under aseptic condition with constant shaking. Then, they were washed three times with sterilized distilled water. The explants were then inoculated aseptically into MS media supplemented with different concentrations and combinations of growth regulators (Table 2).

The culture tubes set were kept at 25 ± 1°C, RH - 70% and 16 h light (2000 to 3000 lux) and 8 h dark period condition in the culture room. Visual observation of culture tubes was made every week and data were recorded after first week of culture.

RESULTS

Among the explants used for the culture, nodal parts did not respond to form callus. Only leaf explants responded to produce profuse calli.

The results of responses of different hormonal concentrations on the callus in MS media of the leaf segments of *I. obscura* are presented in Tables 1 and 2. The highest amount of callus was found in the MS medium with 10.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4 D) with the combination of 10.0 mg/l benzylaminopurine (BAP). The lowest amount of callus was found in 1.25 mg/l indole-3-butyric acid (IBA) with combination of 10.0 mg/l BAP. It is interesting to note that 2,4-D in combination (0.5 to 10.0 mg/l) with BAP (10.0 mg/l), formed high percentage of calli (Table 1). In the case of indole-3-acetic acid (IAA) (0.5 to 10.0 mg/l) and BAP (5.0 and 10.0 mg/l) combination, it produced moderately high callus. Whereas, naphthaleneacetic acid (NAA) (0.5 to 10.0 mg/l) in combinations with BAP (1.25, 0.5 and 10.0 mg/l) gave better response in callus induction.

In the case of rooting response, it was found that IAA formed (1.25 to 10.0 mg/l) roots within 31 to 36 days and

Table 2. Production of *I. obscura* (L.) callus from leaf explants in different concentrations of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D).

IAA (mg/l)	IBA (mg/l)	NAA (mg/l)	2,4 D (mg/l)	Day for root initiation	Length of root (mm)*	Response of rooting (%)**	Day to callus induction	Colour of callus	Nature of callus
0.5				____	0.00	____	____	Nil	Nil
1.25				36	8.33 ± 1.15	53.3 ± 2.0	15	Slightly green	Medium
2.5				33	8.33 ± 1.15	61.3 ± 1.52	15	Brownish	Compact, medium
5.0				32	9.0 ± 1.0	60.66 ± 1.52	13	Whitish brown	Compact, medium
10.0				31	11.0 ± 2.0	63.33 ± 1.52	12	White	Compact, large
	0.5			____	0.00	0.00	17	White	Compact, small
	1.25			____	0.00	0.00	17	White	Compact, small
	2.5			____	0.00	0.00	18	White	Friable
	5.0			____	0.00	0.00	19	White	Friable
	10.0			37	6.1 ± 0.20	9.33 ± 0.57	21	White	Friable, large
		0.5		45	9.0 ± 2.0	52.0 ± 1.0	12	Greenish	Compact, large
		1.25		43	13.66 ± 3.05	63.33 ± 2.08	12	Greenish white	Friable, large
		2.5		42	14.0 ± 2.0	65.33 ± 2.51	12	Whitish brown	Compact, small
		5.0		41	13.33 ± 2.51	69.0 ± 1.0	08	Whitish green	Compact, small
		10.0		25	19.0 ± 2.0	73.66 ± 2.08	11	Whitish green	Compact, small
			0.5	____	0.00	0.00	16	Whitish green	Compact, small
			1.25	____	0.00	0.00	15	Whitish green	Compact, small
			2.5	____	0.00	0.00	15	Whitish green	Compact, large
			5.0	____	0.00	0.00	13	White	Compact, large
			10.0	____	0.00	0.00	12	White	Compact, large

* Average response of three replicates of root length from callus and data (± SD) were recorded up to five weeks of culture. **Average percentage of rooting response of three replicates from callus and data (± SD) were recorded up to five weeks of culture. (–) Indicate no root initiation and callus induction.

NAA formed roots (0.5 to 10.0 mg/l) within 25 to 45 days (Table 2). But 2,4-D did not respond in root initiation in any concentration. Exceptionally, IBA with 10.0 mg/l concentration initiated root formation within 37 days (Table 2).

DISCUSSION

The hormonal content of a culture medium is crucial forany sustained growth of the culture (Narayanaswamy, 2005). The growth and development of higher tissues *in vitro*, especially during callogenesis in various concen-tration mixtures, is controlled by gradients of endogenous plant growth substances (Narayanaswamy, 2005). The ratio of auxins and cytokinins influences the balance between root and shoot formation. Cytokinins inhibit root formation as evidenced during callogenesis.

Axenically grown cotyledonary leaf segments exposed to various concentrations of IAA (0.5 to 10.0 mg/l) and BAP (0.5 to 10.0 mg/l) produced profuse calli (Figure 2-3) (Table 2). BAP is commonly used than kinetin for inducing rapid multiplication of shoot buds or meristems (5 to 50 mg/l). But the response pattern is unique and contrary to usual response. In this case, a high amount of BAP (10.0 mg/l) was found indispensible to evoke callogenesis in all combinations of IAA (0.5 to 10.0 mg/l). The best response was found to be about 5.0 and 10.0 mg/l for IAA and BAP, respectively.

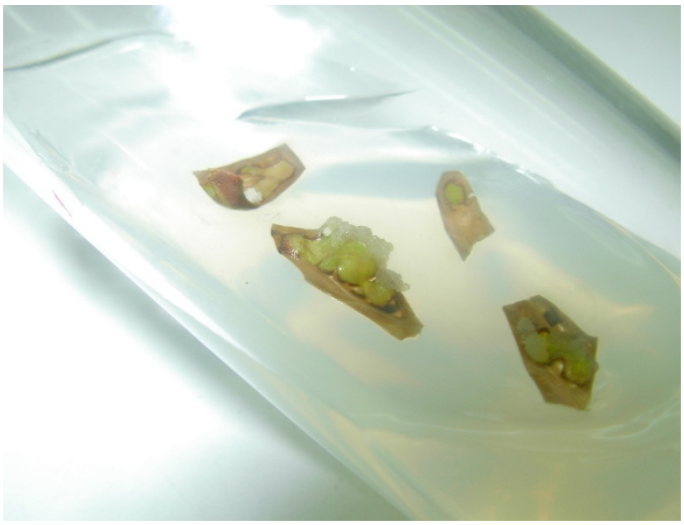

Figure 1. Callogenesis by cotyledonary leaf explants after 7 days (naphthaleneacetic acid (NAA) 2.5 mg/l, benzylaminopurine (BAP) 10.0 mg/l).

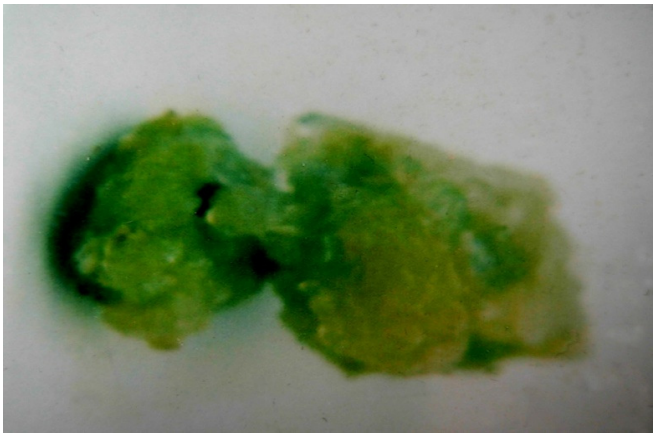

Figure 2. Callogenesis by cotyledonary leaf explants after 15 days.

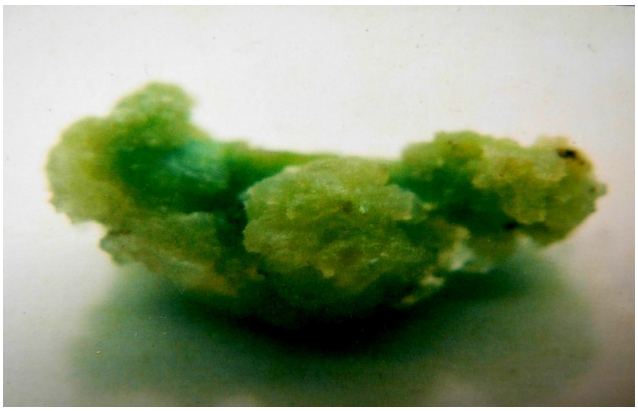

Figure 3. Callogenesis of cotyledonary leaf explants after 15 days (indole-3-acetic acid (IAA) 2.5 mg/l, benzylaminopurine (BAP) 2.5 mg/l).

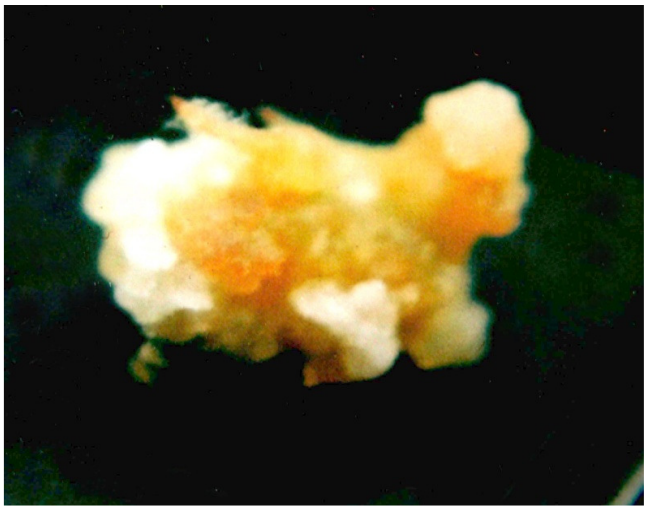

Figure 4. Callogenesis by cotyledonary leaf explants after 15 days (indole-3-butyric acid (IBA) 5.0 mg/l, benzylaminopurine (BAP) 1.25 mg/l)

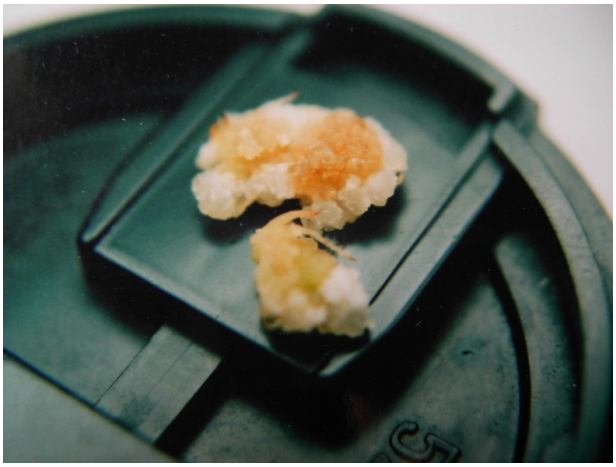

Figure 5. Rooting from callus of cotyledonary leaf explants after 15 days (2,4-dichlorophenoxyacetic acid (2,4-D) 10.0 mg/l, benzylaminopurine (BAP) 5.0 mg/l).

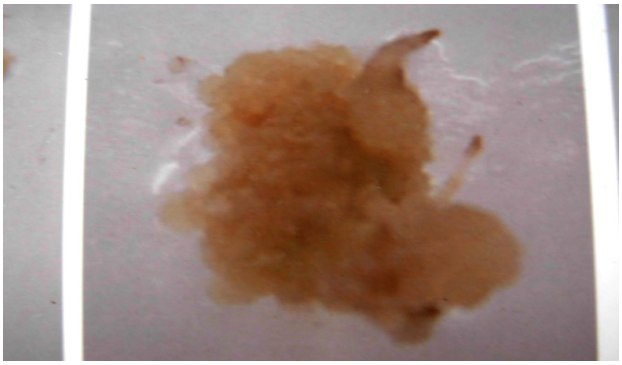

Figure 6. Rooting from friable callus of cotyledonary leaf explants after 15 days (indole-3-butyric acid (IBA) 5.0 mg/l, benzylaminopurine (BAP) 1.25 mg/l).

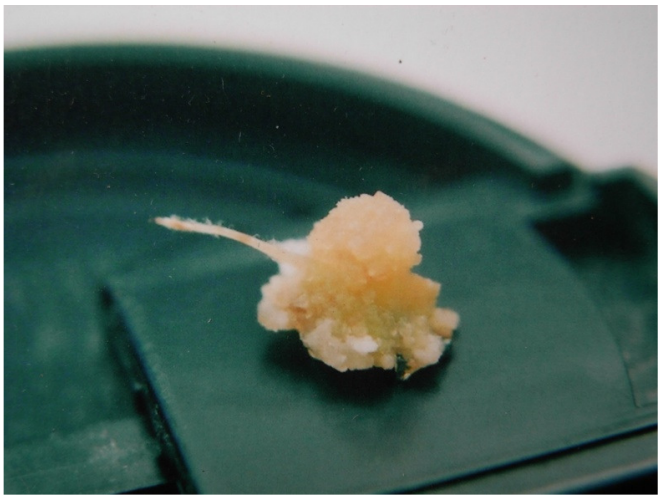

Figure 7. Rooting from friable callus after 15 days (naphthaleneacetic acid (NAA) 10.0 mg/l, benzylaminopurine (BAP) 10.0 mg/l).

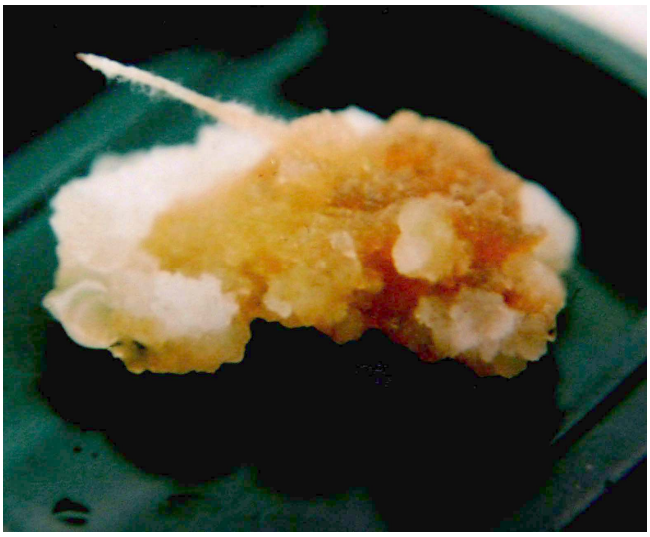

Figure 8. Rooting from friable callus after 25 days (naphthaleneacetic acid (NAA) 10.0 mg/l, benzylaminopurine (BAP) 10.0 mg/l).

In general, relatively high concentrations of auxin suppress organized growth and promote the formations of meristem-like cells. In our study, IAA was used for callus induction (1 to 10 mg/l) that promoted organogenesis. The effect of different concentration of only IAA in general favored callogenesis. In all cases, it took more or less than 13 to 15 days for callus induction. Still, higher concentration of IAA (10.0 mg/l) produced vigorous, white and compact calli (Table 2). The combination of BAP and 2,4-D stimulated better callus formation. Here, even a low concentration of 2,4-D (0.5 mg/l) with BAP (5.0 to 10.0 mg/l) was found to induce effective callogenesis. On the other hand, a moderate to high concentration of both BAP (2.5, 5.0 and 10 mg/l) and 2.4-D (5.0 and 10.0 mg/l)

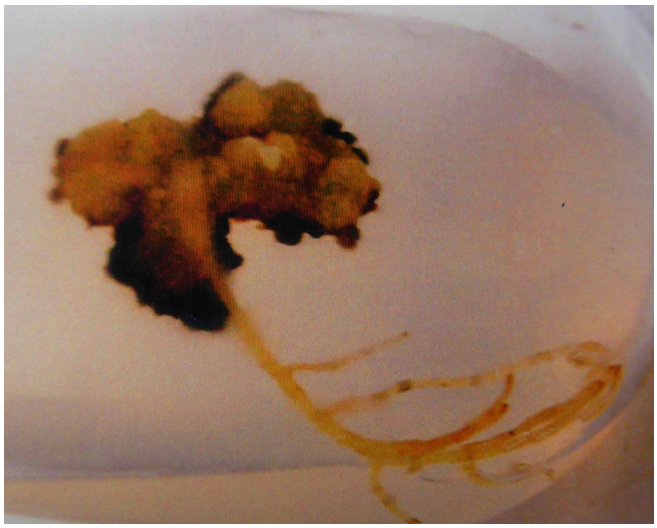

Figure 9. Rooting from friable callus after 40 days (naphthaleneacetic acid (NAA) 10.0 mg/l, benzylaminopurine (BAP) 10.0 mg/l), callus showing production of phenols.

was found to stimulate appreciable callus induction with hairy roots Figure 5 (Table 1).

Moreover, it was noticed that IBA (0.5 to 10.0 mg/l) failed to induce callogenesis with axenically grown leaf segment in *I. obscura.* But with 2,4-D (0.5 and 10.0 mg/l), it took an average of 15 days to produce compact whitish green embryogenic calli. Interestingly, in this case, 2,4-D acts more as an embryogenic stimulant leading to tissue differentiation (Table 2).

The combination, NAA and BAP was almost similar with the results of IAA and BAP in inducing callogenesis (Table 1). In general, NAA (2.50 to 10.0 mg/l), were found to be callus inducing (Table 1). Interestingly, unlike IAA (Table 2) , NAA initiated rooting from friable enlarged whitish green calli (previously raised with 2,4-D at 10 mg/l) within 10 to 12 days in all concentrations (0.5 to 10.0 mg/l) tried. This NAA induced rhizogenesis from friable calli reflected the conventional effect of IBA stimulated rooting (Table 2).

Combination of IBA with BAP was found to be less encouraging for callus formation. Slight callogenesis with root was noticed in higher concentration of both IBA (5.0mg/l) and BAP (10.0mg/l) (Figure 4&6) (Table1). IBA alone induced rooting, the leaf segments of *I. obscura* did not respond at all. Contrarily, small clumps of white compact undifferentiated calli generated after 19 days of culture. It indicates that IBA is a poor calli inducer and has little ability to initiate rhizogenesis.

Considering all the findings, it is evident that, with regard to callogenesis, relatively high concentration (10 mg/l) of both NAA and BAP was essential for rooting response Figure 7-8. Furthermore, as regards rooting response, NAA and BAP induced callus showed emergence of roots only when the callus became old (31 days) (Figure 9). But, better rooting response was

noticed when, 2,4-D induced green friable callus was subcultured in the same concentration (10 mg/l) of NAA and BAP. The results seem to support further micropropagation study of the promising medicinal plant.

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