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Mass propagation of an economically important medicinal plant, L*obelia nicotianaefolia* Heyne using *in vitro* culture technique

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ABSTRACT

An efficient protocol was developed to initiate *in vitro* propagation from leaf explants of the important medicinal plant species, *Lobelia nicotianaefolia* inhabiting the open habitates of Nilgiris, the Western Ghats at high altitudes. The MS medium supplemented with growth hormones NAA and Kn at the concentration of 2.5 and 1.0mg/l respectively is determined to be the optimum for higher frequency of callus formation from leaf explants. Maximum number of shoots (9 shoots/callus) was observed in MS medium fortified with BAP and TDZ at each 1.5mg/l respectively. MS medium with IBA at 0.5.mg/l alone produced higher number of roots during subculturing (12 roots/callus). The plantlets obtained were successfully transferred in the hardening medium containing red soil, coir waste and vermicomposte in the ratio of 1:1:1 by volume in which 88% survivability was achieved. © 2012 Trade Science Inc. - INDIA

KEYWORDS

Lobelia nicotianaefolia;
Medicinal plant;
Nilgiris;
The Western Ghats.

INTRODUCTION

At global level still over 70% of the people rely medicinal plants for their health care system. Megabiodiversity countries including India are considered to be the potential nations for medicinal plants. India possesses rich floristic wealth and diversified genetic resources of medicinal plants. *Lobelia nicotianaefolia* Heyne, a well known medicinal plant inhabiting the Nilgiris(>1800m above msl), India has been used to treat a variety of diseases like asthma, bronchitis, coughs pneumonia, cold and flue, and other upper respiratory problems[25] and has antioxidant properties also[15]. One of the alkaloids in lobelia plant, lobeline has the effect on humans similar to those of nicotine and can be helpful in treating the symptoms of nicotine withdrawl. It may be used to treat hangovers and alcoholism[8]. Owing to these uses, overexploitation is an inevitable reality concerning this species and due to which it is decreasing in population size in its habitats of distribution in Nilgiris of Western Ghats, India[15, 16]. The conventional method of propagation of this species is mainly through seeds. However, the poor germination potential of seeds restricts its multiplication to considerable extent[17]. Hence, tissue culture technique offers an alternative method to propagate this species. This research describes the *in vitro* regeneration of *L. nicotianaefolia* from leaf discs by employing tissue culture techniques and successful establishment of this species in soil.

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MATERIALS AND METHODS

The leaf explants of *Lobelia nicotianaefolia* were collected from the healthy individuals at Nilgiris and washed thoroughly with tap water, then they were cut into small discs of 0.8cm diameter and then treated with a surfactant, tween 20 (5% w/v) for 5 minutes. After repeated washes in double distilled water, to eliminate the fungal contamination, the explants were treated with Carbendazim (50% w/v) fungicide (10%) also for 15 minutes and rinsed with double distilled water 2 or 3 times. To eliminate bacterial contamination, explants were also treated with 5% antibiotics (ampicillin and rifampicin) for 30 minutes followed by three rinses in sterile double distilled water. Furthermore, surface sterilization was carried out by dipping the explants in 0.1% $HgCl_2$ for 3 minutes followed by 3-4 rinses in sterilized double distilled water inside the Laminor air flow chamber.

Leaf discs were horizontally placed in Petri dishes containing MS[14] medium fortified with various combinations and concentrations of different growth regulators *viz.*, BAP, NAA, 2,4-D, Kn and TDZ for callus induction. The pH of the medium was adjusted between 5.6 and 5.8 before autoclaving at 121°C for 20 min. The culture was incubated at a constant temperature of 25±2°C with 14h photoperiod (3000 lux) and 8h darkness. Callus from these primary cultures were transferred to MS medium containing different concentrations of BAP, TDZ and GA_3 for shoot induction. After the origin of multiple shoots, elongated shoots of 2 cm long were excised from the culture and transferred to MS medium supplemented with different concentrations of IBA, IAAand NAA for root initiation. After two weeks, the percentage of shoot forming roots and roots per shoot length were assessed. Rooted shoots were thoroughly washed to remove the adhering gel, and planted in polythene bags containing different hardening media and kept in greenhouse for acclimatization. The pots were watered at one day interval and supplied with ½ strength MS salts, twice a week by spraying. The survival rate of plantlets was recorded one month after transfer to polythene bags. Triplicates were maintained for all experiments.

RESULTS AND DISCUSSION

The number of days required for callus induction from the leaf explants of the study species, *Lobelia nicotianaefolia* is noted to be varied from 15 to 25 days according to the combinations and concentrations of the growth regulators used *viz.*, BAP, NAA, 2,4-D, Kn and TDZ in the MS medium (TABLE 1). It may be explained that the specific growth hormones at appropriate concentrations can play major role to induce callus besides the other factors[1]. The amount of leaf ex-

TABLE 1 : Effect of different combinations and concentrations of growth regulators on callus formation from leaf explants of *Lobelia nicotianaefolia.*

Growth regulator (mg/L)					Days required for callus formation	Callus formation (%)	Colour of the callus
BAP	NAA	2,4-D	Kn	TDZ			
0.5	0.0	0.0	0.0	0.0	-	-	G
1.0	0.0	0.0	0.0	0.0	15	11.33^{a}±1.52	G
1.5	0.0	0.0	0.0	0.0	17	23.67^{b}±1.52	G
2.5	0.0	0.0	0.0	0.0	19	32.67^{c}±1.52	G
3.0	0.0	0.0	0.0	0.0	20	37.00^{ce}±1.00	G
0.0	0.5	0.0	0.3	0.0	23	50.00^{d}±1.00	G
0.0	1.0	0.0	0.5	0.0	24	60.00^{e}±1.00	G
0.0	1.5	0.0	0.7	0.0	18	70.00^{f}±1.00	DG
0.0	2.0	0.0	0.9	0.0	20	80.00^{g}±1.00	DG
0.0	2.5	0.0	1.0	0.0	25	95.66^{h}±2.51	DG
0.5	0.0	0.4	0.0	0.0	19	30.33^{c}±1.52	G
1.0	0.0	0.8	0.0	0.0	17	33.33^{c} ±2.08	DG
1.5	0.0	1.2	0.0	0.0	21	40.33^{e} ±1.52	DG
2.0	0.0	1.6	0.0	0.0	23	46.00^{d}±2.00	LG
2.5	0.0	2.0	0.0	0.0	22	50.33^{d}±1.52	LG
3.0	0.0	2.4	0.0	0.0	20	10.33^{a}±1.52	B
0.0	0.5	0.0	0.2	0.0	17	20.00^{b}±1.00	LG
0.0	0.5	0.0	0.4	0.0	18	22.33^{b}±2.08	LG
0.0	0.5	0.0	0.6	0.0	21	27.33^{b}±1.52	B
0.0	0.5	0.0	0.8	0.0	24	37.00^{c}±2.00	LB
0.0	0.5	0.0	1.0	0.0	22	40.00^{e}±1.00	LB
0.0	0.3	0.0	0.0	0.1	23	36.66^{ce}±1.52	DB
0.0	0.3	0.0	0.0	0.2	21	29.33^{c}±1.52	DB
0.0	0.3	0.0	0.0	0.3	19	26.67^{ci}±1.54	DB
0.0	0.3	0.0	0.0	0.4	20	24.66^{bi}±1.52	DB
0.0	0.3	0.0	0.0	0.5	24	23.33^{b}±2.08	LB

G-Green, DG- Dark green, LG- Light green, B-Brown, DB-Dark brown, LB- Light brown; Means in column followed by different letter(s) are significantly different at 5% level according to DMRT.

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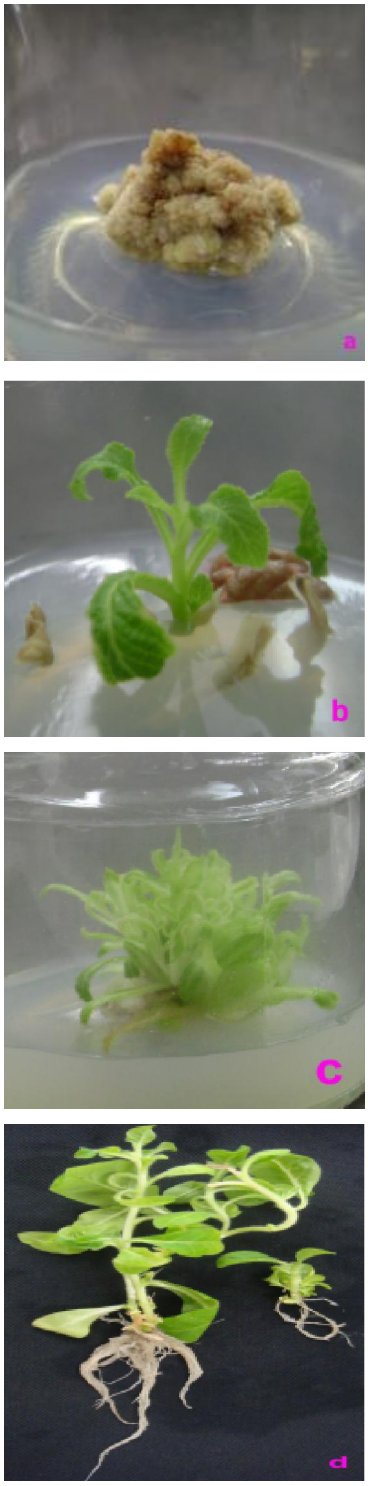

Figure 1 : Successful *in vitro* culture of *Lobelia nicotianaefolia* from the leaf explants. a, Effective callus formation in MS medium containing NAA and Kn at 2.5 mg/l and 1.0 mg/l; b, Induction of shoots in leaf derived callus on MS medium fortified with BAP and GA_3 at 2.5 mg/l and 0.5 mg/l; c, Multiple shoot regeneration form leaf segments on MS medium supplemented with BAP and TDZ at 1.5 mg/l respectively; d, Rooting of shoots on MS medium containing 0.5 mg/l of IBA.

plant responding for callus formation was ranging between 10 and 95% (TABLE 1). MS medium fortified with NAA and Kn at 2.5 and 1.0 mg/l initiated 95.66% of leaf discs for callus formation (Figure 1a) followed by 2.0 and 0.9 mg/l of NAA and Kn which initiated 80% of leaf discs for callusing and 1.5 and 0.7mg/l of NAA, and Kn each initiated 70% of discs for callusing. The other combinations and concentrations of growth hormones in the medium initiated around only 20 to 50% of leaf discs of *L.nicotianaefolia* for callus formation. Baskaran and Jayabalan[2] explained that the differential response of same or different explants for callus formation could be due to the nature of tissue, degree of totipotency and composition of medium with respect to micronutrients and hormones. Further it is explained that the variation in response of discs in terms of callus initiation may be due to the variation in distribution of endogenous level of growth regulators as observed in many other plants[2, 4, 5, 6, 21, 23]. It was noted that the NAA alone or in combination with Kn generally have the efficiency of initiation at high percentage of leaf explant (>50%) for callus formation. It indicates the higher requirement of certain auxins like NAA in combination with low quantity of cytokinins like Kn for callus formation of the study species, *L.nicotianaefolia.* Karappusamy and Pullaiah (2007) for the species, *Bupleurum distichophyllum* and[20] for the species, *Ageratum conyzoides* also reported effective callus formation from the leaf explant in the medium containing high quantity of NAA. Mariani et al.,[12] reported the requirement of the cytokinin like compounds for effective callus formation in the ornamental plant, *Aglaonema* sp. The colour of the calli was showing wide degree like green, dark green, light green, brown, dark brown and light brown according to the combinations and concentrations of the growth regulators in the MS medium (TABLE 1).

The results of the subculturing experiments by using the secondary explant, leaf derived callus showed that the cytokinin, BAP (2.5 mg/l) in combination with GA_3 (0.5 mg/l) enhanced the response of calli for shoot formation by 98 and 86% respectively (TABLE 2) (Figure 1b). In addition, greater number of 9 shoots/callus was also noted to be produced while subculturing the calli on MS medium with BAP and TDZ each at 1.5 mg/l respectively (TABLE 2) (Figure 1c). However,

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TABLE 2 : Effect of different combinations and concentrations of growth regulators on shoot initiation, shoot number and shoot length after subculturing the leaf derived callus of *Lobelia nicotianaefolia*

Growth regulator (mg/L)			Culture Response (%)	No. of shoots /callus	Shoot Length (cm)
BAP	TDZ	GA_3			
0.1	0.1	0.0	08.00 [a]±1.00	1.67 [a]±1.15	2.23 [a]±0.16
0.2	0.2	0.0	12.00 [b]±1.00	3.00 [b]±1.00	3.33 [b]±0.56
0.3	0.3	0.0	16.33 [c]±1.53	2.00 [a]±1.00	1.36 [c]±0.19
0.4	0.4	0.0	20.00 [d]±1.00	1.68 [a]±0.58	3.90 [b]±0.26
0.5	0.5	0.0	30.00 [e]±1.00	2.00 [b]±1.00	4.46 [d]±0.89
0.6	0.6	0.0	34.00 [e]±1.00	3.00 [c]±1.05	3.47 [b]±0.45
0.7	0.7	0.0	38.67 [f]±1.53	3.68 [d]±1.52	2.83 [a]±0.49
0.8	0.8	0.0	41.33 [f]±1.53	3.33 [ce]±1.54	4.66 [d]±0.16
0.9	0.9	0.0	46.00 [g]±1.00	4.00 [d]±1.08	5.50 [d]±4.84
1.0	1.0	0.0	50.00 [g]±2.00	4.66 [f]±1.56	7.56 [e]±0.25
1.1	1.1	0.0	60.00 [h]±1.00	5.66 [g]±1.48	4.90 [d]±0.10
1.2	1.2	0.0	70.00 [i]±1.00	5.33 [g]±0.58	2.30 [a]±0.20
1.3	1.3	0.0	80.00 [j]±1.00	6.67 [h]±1.59	3.63 [b]±0.65
1.4	1.4	0.0	90.00 [k]±1.00	6.33 [h]±1.68	4.86 [d]±0.19
1.5	1.5	0.0	95.67 [k]±2.08	9.69 [j]±1.29	6.33 [f]±0.40
1.6	1.6	0.0	81.33 [j]±1.53	7.00 [h]±1.00	5.40 [d]±0.29
1.7	1.7	0.0	70.00 [i]±1.00	8.00 [j]±1.00	4.10 [d]±0.20
1.8	1.8	0.0	51.33 [g]±1.53	7.33 [j]±1.54	3.60 [b]±0.20
1.9	1.9	0.0	54.33 [g]±1.53	7.01 [j]±1.00	2.90 [a]±0.55
2.0	2.0	0.0	56.67 [h]±1.53	8.05 [j]±1.02	1.70 [c]±0.52
0.5	0.0	0.1	63.67 [h]±1.53	8.69 [k]±1.26	3.30 [b]±0.20
1.0	0.0	0.2	70.00 [i]±1.00	8.50 [k]±1.96	4.53 [d]±0.30
1.5	0.0	0.3	80.00 [j]±1.00	8.36 [k]±1.64	5.26 [d]±0.68
2.0	0.0	0.4	86.33 [k]±1.53	7.00 [j]±1.00	4.10 [d]±0.40
2.5	0.0	0.5	98.00 [l]±1.00	8.68 [k]±1.06	6.00 [f]±0.72

Means in column followed by different letter(s) are significantly different at 5% level according to DMRT.

TABLE 3 : Effect of different combinations and concentrations of growth regulators on root number rooting, percentage and root length after subculturing the leaf calli derived shoots of *Lobelia nicotianaefolia.*

Growth Regulator (mg/L)			Shoots Rooted (%)	No. of roots/ shoot	Root Length (cm)
IBA	IAA	NAA			
0.1	0.0	0.0	08.00 [a] ±1.00	07.35 [a]±1.45	2.16 [a]±0.15
0.2	0.0	0.0	11.67 [b] ±1.16	09.33 [b]±1.53	2.63 [a]±1.53
0.3	0.0	0.0	16.00 [c]±1.00	10.35 [bc]±1.62	3.00 [a]±0.10
0.4	0.0	0.0	80.33 [di]±4.72	11.38 [cd]±1.68	7.20 [b]±0.17
0.5	0.0	0.0	87.00 [d]±1.00	12.00 [d]±1.00	7.70 [be]±0.28
0.6	0.0	0.0	83.00 [d]±2.65	10.33 [bc]±1.68	6.83 [b]±0.58
0.7	0.0	0.0	38.67 [e]±1.56	10.33 [bc]±1.62	5.63 [c]±0.15
0.8	0.0	0.0	41.00 [e]±1.75	07.69 [a]±1.65	6.30 [b]±0.20
0.9	0.0	0.0	47.00 [f]±2.00	08.33 [a]±1.25	7.10 [be]±0.30
1.0	0.0	0.0	50.00 [f]±2.00	06.33 [e]±1.56	7.66 [be]±0.15
0.0	0.1	0.0	60.00 [g]±1.00	05.00 [f]±2.00	6.50 [b]±0.17
0.0	0.2	0.0	67.33 [h]±2.08	07.00 [a]±2.00	7.10 [b]±0.19
0.0	0.3	0.0	76.67 [i]±3.51	09.33 [b]±1.68	7.50 [be]±0.16
0.0	0.4	0.0	90.00 [j]±2.68	04.00 [g]±1.00	3.33 [a]±0.25
0.0	0.5	0.0	95.00 [j]±3.05	05.67 [f]±1.61	3.30 [a]±0.05
0.0	0.6	0.0	93.67 [j]±3.21	06.00 [e]±1.00	3.80 [a]±0.26
0.0	0.7	0.0	70.00 [h]±1.00	09.35 [b]±1.21	6.33 [b]±0.15
0.0	0.8	0.0	51.33 [fh]±1.54	08.33 [a]±1.65	7.63 [be]±0.18
0.0	0.9	0.0	54.68 [gh]±1.58	07.35 [a]±1.62	6.60 [b]±0.14
0.0	0.0	0.1	57.68 [g]±2.51	05.10 [f]±1.00	6.70 [b]±0.05
0.0	0.0	0.2	63.66 [g]±1.58	03.67 [g]±1.52	8.00 [de]±0.14
0.0	0.0	0.3	71.00 [h]±2.64	03.00 [g]±1.00	3.20 [a]±0.26
0.0	0.0	0.4	20.00 [c]±1.00	09.36 [b]±1.26	3.73 [a]±0.15
0.0	0.0	0.5	31.33 [k]±2.51	11.00 [cd]±2.64	4.80 [c]±0.10
0.0	0.0	0.6	34.00 [k]±1.00	08.00 [a]±1.00	5.10 [c]±0.26

Means in column followed by different letter(s) are significantly different at 5% level according to DMRT.

the higher shoot length of 7.56cm was achieved in the MS medium fortified with BAP and TDZ each at 1.0 mg/l (TABLE 2). All these facts indicate that the cytokinin, BAP is the most essential growth regulator for effective shooting of the study species, *L.nicotianaefolia*. It is of common fact that cytokinin is the major growth hormone involved in shoot formation in many plant species[19, 20, 22, 24].

The rooting attributes of *L.nicotianaefolia* while subculturing the secondary explant shoots were well pronounced in the MS medium supplemented with the auxin, IAA alone at higher concentrations from 0.1 to 0.9 mg/l (TABLE 3). The IAA concentration at 0.5 mg/l initiated 95% shoots for root formation (Figure 1d) followed by 0.6mg/l initiated 93% and 0.4 mg/l initiated 90% shoots for root formation. The number of roots per shoot was also observed to be higher (12 roots/shoot) in the MS medium containing 0.5mg/l IBA for the study species *L.nicotianaefolia.* Similarly, the root length was greater (8cm) during the subculturing of *in vitro* cultured shoots for roots on MS medium with NAA at 0.2 mg/l. All these findings showed that the auxin, IAA is the most required growth regulator for shooting characters of the study species,

L.nicotianaefolia. It agrees with the concept that auxins are the plant hormones endogenously or exogenously inducing root formation in majority of plant species[23]. Similar kind of findings of effective root formation by the influence of various types of auxins in many plant species have been reported elsewhere[9, 10, 11, 13, 18].

The hardening experiments showed that high degree of acclimatization was achieved by performing 88% of plantlet survivability in the hardening medium encomposed by red soil, coirwaste and vermicompost in the ratio of 1:1:1 by volume. Hence, before transplanting the plantlets, hardening must be done in this prescribed encomposed medium for higher survivability of plantlets. However, field observations can be made in the habitatess of higher altitudes of Nilgiris after transplantation to know the rate of survivability in the open environmental conditions.

ABBREVIATIONS

2, 4-D	2, 4-Dichlorophenoxyacetic acid
BAP	6-Benzyladenine
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
Kn	Kinetin
NAA	Naphthaleneacetic acid
TDZ	Thiodizuron
GA_3	Gibberlic acid

CONCLUSION

The application of plant tissue culture is one of the alternative propagation method for large production of selected germplasm ensuring genetic stability, continuity and uniformity. Due to the medicinal and botanical importance, the plant species *L.nicotianaefolia*, continuously exploited by the tribals, local public, researchers and herb gatherers and so it is facing constant threat. As the studied medicinal plant seems to have high potential in drug industry, to achieve bulk production this prescribed protocol can be used.

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