

***In vitro* Mass Propagation Protocol for *Lobelia chinensis* Lour.: Optimization of Growth Regulators and Acclimatization**

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Abstract

A reliable *in vitro* regeneration protocol has been developed for *Lobelia chinensis* Lour., an important medicinal plant with limited availability in Bangladesh. Nodal explants were cultured on MS medium with different cytokinins and auxins. The highest regeneration response (90%) was obtained on MS medium containing 5.55 µm/l BAP + 0.56 µm/l NAA, producing 14.00 ± 1.0 shoots per explant with a mean shoot length of 3.83 ± 0.17 cm within 28-42 days. In contrast, PGR-free MS medium showed only 20% response with 3.17 ± 0.44 shoots per explant and 1.17 ± 0.17 cm shoot length. The best rooting response (99%) and root per explant (29.33 ± 0.67) occurred in MS medium with 5.55 µm/l NAA within 14-21 days. While maximum root length (2.83 ± 0.17 cm) was observed in MS + 8.33 µm/l NAA. Regenerated plantlets were successfully acclimatized in a 1 : 1 mixture of garden soil and compost with 90% survival.

Introduction

Lobelia chinensis is a genus of the plant family Campanulaceae, which includes more than 430 species and exhibits diverse habitats in tropical, subtropical and temperate sites worldwide (John et al. 2020). The largest centre of diversity of *Lobelia* is in Africa which contains about 37% species, while Asia is inhabited by nearly 12% (John et al. 2020). *L. chinensis*, commonly called Chinese lobelia or creeping lobelia, is a perennial herb that comes from East and Southeast Asia (Sit et al. 2017). Its native range stretches from Bangladesh, China, Indonesia, Japan, Malaysia, Nepal, Srilanka and India (Singh and Diwakar 2010). The species has several local names. In China, it is called "*Ban-bian-lian*." In Japan, it's known as "*Aze-Mushiro*." In Korea, people refer to it as "*Su-yeom-ga-rae-kkot*," and in Bangladesh, it goes by "*Haita ghass*" or "*Hat ful*" (Beom et al. 2021). In Bangladesh six species of the *Lobelia* genus have been officially documented (Islam and Hossain 2008). Although *L. chinensis* has been reported in the area, it is rare and has not been well studied considering its propagation and conservation. The limited presence of this species in its natural habitat shows the need for developing new methods to

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conserve it and use it sustainably. *L. chinensis* has a long history of use in traditional Chinese medicine. The whole plant is valued for its anti-inflammatory, cleansing, fever-reducing, and diuretic properties. Traditionally people crush fresh plant material and apply it externally as a poultice to treat inflammation, abscesses, insect bites, and injuries (Wiert 2006). In Chinese folk medicine, the plant is also used as an antidote, blood stopper, and treatment for swelling, diarrhea, jaundice, and respiratory issues (Wang et al. 2018). Phytochemical studies show that *L. chinensis* contains several bioactive compounds, including alkaloids like lobeline, which have effects similar to nicotine (Felpin and Lebreton 2004). Because of these properties, lobeline was once included in smoking cessation products, but this use was later limited by the U. S. Food and Drug Administration due to a lack of effectiveness (Wang et al. 2018). Recent pharmacological research has shown that extracts and decoctions of *L. chinensis* have anti-inflammatory, antioxidant, antiviral, antimicrobial, anti-tuberculosis, anticancer, and antitumor properties (Zhang et al. 2018, Jo et al. 2021). The extract of the plant when heated has proven to stimulate the immune system and has demonstrated anticancer activity against models of gastric and liver cancers (Zhang et al. 2018). *L. chinensis*, although considered medicinally valuable, is experiencing increased pressure from overharvest, habitat destruction, slow natural growth rate, and vulnerability to insect pests and pathogens (Joshi et al. 2011). It is commonly viewed as a weed and is found more frequently in cultivated or disturbed areas than in their original habitat. Because of this, it is critical to develop conservation-based propagation strategies to support sustainable use of these plants. One way to do this is to utilize plant tissue culture techniques as a means of conserving, propagating, and sustainably using this medicinal plants. Rapid clonal multiplication and pathogen-free planting material production are accomplished through the utilization of *in vitro* methods (Reddy 2011). Some investigators have reported *in vitro* culture for *L. chinensis* and regenerative capacity in other areas of the world (Bai et al. 2023, 2024). However, there is no information available regarding the *in vitro* propagation of this plant from Bangladesh. The present study aimed to develop an efficient and reproducible *in vitro* regeneration protocol for *L. chinensis* using nodal explants. This study evaluated the effects of plant growth regulators on shoot and root induction and establishing a reliable acclimatization method. These findings will also assist in conserving and sustainable utilization of *L. chinensis*, moreover contribute to the pharmacological research particularly by using the *in vitro* grown plants.

Materials and Methods

Healthy plants of *L. chinensis* were chosen for *in vitro* experiments (Fig. 1A). Plants were collected from Chittagong University campus. Nodal segments (0.5-1.0 cm) on selected plants were taken as explants for *in vitro* culture. Collected explants were thoroughly washed with running tap water for about 30 min to remove the external contaminants adhere to the explants. The explants were then deeped into 5% (v/v) savlon solution [Cetrimide (3.0% w/v) and Chlorhexidine Gluconate (0.3% w/v)], and shacken for 10 min

and washed with distilled water. Then the explants were treated with 10% (v/v) Clorox™ for about 10 min and washed thoroughly with distilled water. The next tasks were done in the laminar airflow cabinet. After a 1 min treatment with 70% ethanol, the explants were surface sterilized with an aqueous solution of 0.1% (w/v) HgCl₂ for 10 min. Finally the explants were washed with autoclaved distilled water for 4-6 times for removing the trace of HgCl₂. Explants were then cut into segments (0.5-1.0 cm) restoring the nodal parts and aseptically cultured on 0.8% (w/v) agar solidified MS medium fortified with different plant growth regulators such as BAP (2.78-11.10 µm/l), Kn (2.78-11.10 µm/l), NAA, IAA, IBA (5.55-2.78 µm/l). Before put into culture tubes the pH of the culture media was adjusted to 5.8 and autoclaved at 121°C and 1.1 Kg/cm² for 15 min. All culture vessels were incubated in a culture room at 25 ± 2°C under 16/8 hrs. Photoperiod with a light intensity of 2000-3000 lux. At every stage of the culture, all equipment such as beakers, forceps, filter paper, petri dish, etc., was properly sterilized before use. The aseptic cultures were subcultured after 14 to 21 days on MS medium supplemented with the same or different concentrations of cytokinin and auxin, including BAP, NAA, IAA and IBA. The proliferated shoots were carefully divided into clusters of 2 to 3 shoot tips for incubation. The same processes were repeated 2-3 times to assess and calculate the growth rate. For rooting, elongated shoots (2-2.5 cm) were rescued aseptically from the culture medium and then individually transferred to freshly prepared full and half-strength MS medium, supplemented with different concentrations of NAA, IBA, and IAA. The adventitious roots were produced from the base of the cutting edges of microshoots within 2-3 weeks of culture on a suitable medium.

After sufficient growth, healthy, expanded, and rooted plantlets were gradually acclimatized to natural environmental conditions. Roots were carefully washed to remove the residual agar from culture. The regenerated plantlets were placed in seedling trays with coco peat and left for one week in growth room covered by transparent polythene to prevent evaporation. After one week seedling tray were moved to outside the growth room and gradually acclimatized in natural environment

Statistical analysis was carried out using the statistical program OPSTAT. A completely randomized design (CRD) was adopted to calculate the mean and standard error. Comparisons among means were performed using Duncan's Multiple Range Test (DMRT) for each experiment.

Results and Discussion

Nodal segment underwent direct regeneration on MS medium supplemented with or without various plant growth regulators (Table 1, Fig. 1B). Multiple shoot buds were induced in all treatments with a variation in mean number of shoots per explant and mean length of shoots (Table 1). MS media without plant growth regulators (control) showed poor response regarding per cent response, mean root numbers and mean root length respectively. In cases of PGR suppliments, BAP alone and in combination with NAA proved to be better over the other media tested. It was observed that as the number

Table 1. Effects of different plant growth regulators in MS medium for shoot regeneration from nodal explants of *L. chinensis*.

Cytokinin	Con. ($\mu\text{m/l}$)	Auxin ($\mu\text{m/l}$)	Regeneration (%)	Shoot/explant ($\text{cm} \pm \text{SE}$)	Shoot length ($\text{cm} \pm \text{SE}$)	
Control	-	-	20 \pm 3 ^{lm}	3.17 \pm 0.4 ^f	1.2 \pm 0.2 ^{hi}	
BAP	2.78	-	70 \pm 6 ^{bcd}	9.33 \pm 0.67 ^{bcd}	2.83 \pm 0.60 ^{bcd}	
	5.55	-	83 \pm 3 ^{ab}	10.67 \pm 0.67 ^b	3.33 \pm 0.33 ^{ab}	
	8.33	-	80 \pm 6 ^{abc}	10.0 \pm 1.16 ^{bc}	3.33 \pm 0.33 ^{ab}	
	11.10	-	73 \pm 3 ^{bcd}	9.67 \pm 0.33 ^{bcd}	2.67 \pm 0.33 ^{bcd}	
Kn	2.78	-	63 \pm 3 ^{defg}	7.67 \pm 0.33 ^{cde}	2.50 \pm 0.29 ^{bcd}	
	5.55	-	70 \pm 6 ^{bcd}	9.0 \pm 0.58 ^{bcd}	2.83 \pm 0.44 ^{bcd}	
	8.33	-	70 \pm 0 ^{bcd}	9.33 \pm 0.33 ^{bcd}	2.33 \pm 0.17 ^{cdefg}	
	11.10	-	67 \pm 3 ^{cdef}	8.33 \pm 0.33 ^{bcd}	2.00 \pm 0.29 ^{defghi}	
BAP	2.78	NAA 0.56	80 \pm 0 ^{abc}	13.67 \pm 0.88 ^a	3.17 \pm 0.44 ^{abc}	
	5.55	0.56	90 \pm 0 ^a	14.0 \pm 1.0 ^a	3.83 \pm 0.17 ^a	
	8.33	0.56	80 \pm 0 ^{abc}	13.0 \pm 1.0 ^a	3.17 \pm 0.33 ^{abc}	
	11.10	0.56	73 \pm 3 ^{bcd}	10.33 \pm 0.33 ^b	2.67 \pm 0.17 ^{bcd}	
	2.78	IAA 0.56	43 \pm 3 ^{hijk}	10.0 \pm 1.16 ^{bc}	1.83 \pm 0.33 ^{efghi}	
	5.55	0.56	57 \pm 3 ^{efgh}	10.67 \pm 0.88 ^b	2.17 \pm 0.17 ^{defgh}	
	8.33	0.56	57 \pm 3 ^{efgh}	9.33 \pm 0.33 ^{bcd}	1.70 \pm 0.17 ⁱ	
	11.10	0.56	60 \pm 0 ^{defg}	9.0 \pm 0.58 ^{bcd}	1.33 \pm 0.17 ^{hi}	
	2.78	IBA 0.56	37 \pm 7 ^{jk}	9.33 \pm 0.33 ^{bcd}	1.50 \pm 0.29 ^{ghi}	
	5.55	0.56	40 \pm 0 ^{ijk}	9.33 \pm 0.33 ^{bcd}	2.17 \pm 0.17 ^{defgh}	
	8.33	0.56	40 \pm 6 ^{ijk}	9.67 \pm 0.33 ^{bcd}	1.83 \pm 0.17 ^{efghi}	
	11.10	0.56	33 \pm 3 ^{kl}	8.33 \pm 0.33 ^{bcd}	1.50 \pm 0.50 ^{ghi}	
	Kn	2.78	NAA 0.56	67 \pm 3 ^{cdef}	9.0 \pm 0.58 ^{bcd}	1.83 \pm 0.17 ^{efghi}
		5.55	0.56	70 \pm 6 ^{bcd}	10.0 \pm 0.58 ^{bc}	2.00 \pm 0.29 ^{defghi}
		8.33	0.56	67 \pm 3 ^{cdef}	9.0 \pm 0.58 ^{bcd}	1.67 \pm 0.17 ^{fghi}
		11.10	0.56	63 \pm 3 ^{defg}	9.0 \pm 0.58 ^{bcd}	1.67 \pm 0.17 ^{fghi}
2.78		IAA 0.56	57 \pm 9 ^{efgh}	8.33 \pm 0.88 ^{bcd}	1.67 \pm 0.17 ^{fghi}	
5.55		0.56	63 \pm 3 ^{defg}	8.67 \pm 0.67 ^{bcd}	2.00 \pm 0.29 ^{defghi}	
8.33		0.56	53 \pm 7 ^{fghi}	8.33 \pm 0.88 ^{bcd}	2.00 \pm 0.29 ^{defghi}	
11.10		0.56	53 \pm 7 ^{fghi}	8.33 \pm 0.88 ^{bcd}	1.33 \pm 0.33 ^{hi}	
2.78		IBA 0.56	50 \pm 6 ^{ghij}	7.33 \pm 1.45 ^{de}	1.33 \pm 0.33 ^{hi}	
5.55		0.56	57 \pm 9 ^{efgh}	7.0 \pm 1.53 ^e	2.00 \pm 0.29 ^{defghi}	
8.33		0.56	50 \pm 6 ^{ghij}	9.0 \pm 0.58 ^{bcd}	1.67 \pm 0.17 ^{fghi}	
11.10		0.56	50 \pm 6 ^{ghij}	8.33 \pm 0.33 ^{bcd}	1.67 \pm 0.17 ^{fghi}	

The data are presented as the mean value \pm standard error (SE). Mean values followed by the same letters within the same column are not significantly different based on Duncan's multiple range test conducted at a 95% confidence level.

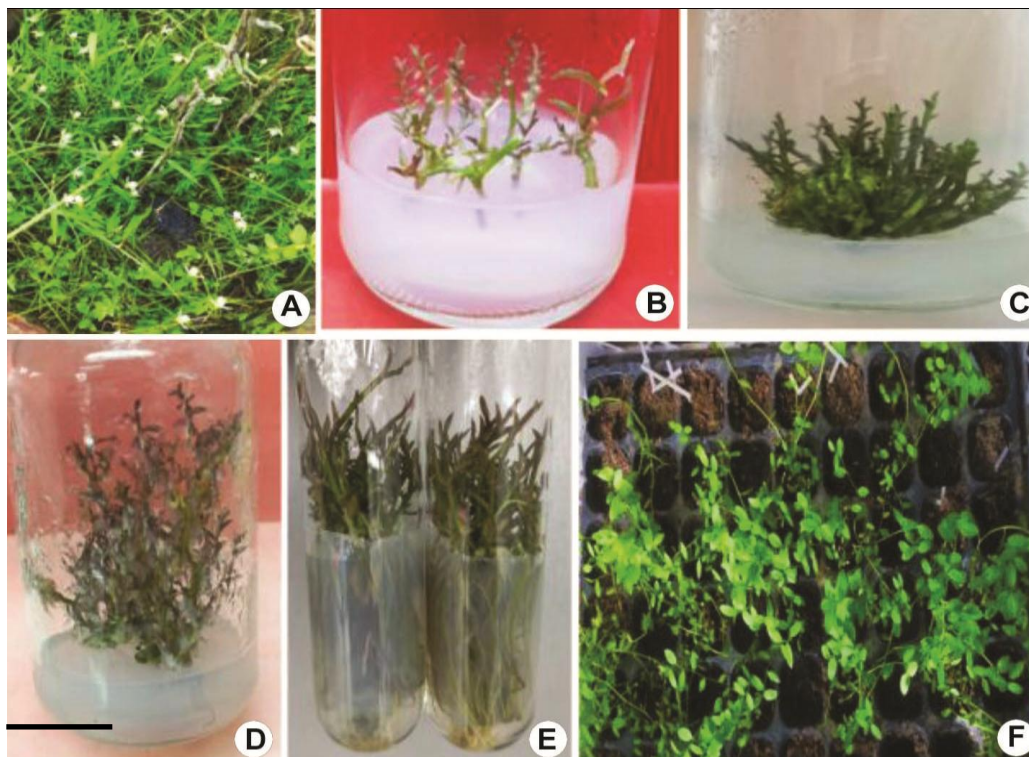


Fig. 1(A-F). *In vitro* propagation of *Lobelia chinensis* from nodal explant: (A) explant source, (B) shoot initiation from nodal explants after 15 days of culture on MS medium containing 5.55 $\mu\text{m/l}$ BAP, (C) shoot multiplication after first subculture at 28 days in 5.55 $\mu\text{m/l}$ BAP + 0.56 $\mu\text{m/l}$ NAA, (D) shoot proliferation and elongation after second subculture at 42 days in 5.55 $\mu\text{m/l}$ BAP + 0.56 $\mu\text{m/l}$ NAA, (E) Development and proliferation of roots in MS with 5.55 $\mu\text{m/l}$ NAA, and (F) acclimatization of regenerated plantlets into seedling tray. Bar: 1 cm.

of subcultures increased, so did the development of multiple shoots and the length of shoot buds. The results of these experiments are given after two subcultures at 14 days interval. When BAP was used singly the highest (83.3%) explants induced multiple shoots with a mean number of shoot (10.67 ± 0.67) and mean shoot length (3.33 ± 0.33 cm) per explant. On the other hand, combination of BAP (5.55 $\mu\text{m/l}$) with NAA (0.56 $\mu\text{m/l}$) significantly enhance the regeneration frequency (90%), mean number of shoot per explant (14.00 ± 1.0) and mean shoot length (3.83 ± 0.17 cm) respectively (Table 1, Fig. 1C). When Kn used singly, the highest shoot regeneration (70%) response was reported in 5.55 and 8.33 $\mu\text{m/l}$. The maximum shoot per explant (9.33 ± 0.33) and length of shoot per explant (2.83 ± 0.44 cm) was recorded in 8.33 and 5.55 $\mu\text{m/l}$ respectively. The efficiency of cytokinin, particularly BAP, has been proved effective than other cytokinins and its combined effect has also been shown effective in *in vitro* plant regeneration (Biswas et al. 2011, Siwach and Gill 2011, Komakech et al. 2020, Paul and Biswas 2024, Nahar et al. 2025). The effectiveness of BAP may result from its ability to stimulate

cell division and promote lateral bud development, which plays an important role in overcoming axillary bud dormancy (Goyal et al. 2015, Sadeghi et al. 2015). They were elongated along with the subcultures (Fig. 1D) in BAP 5.55 $\mu\text{m/l}$ with 0.56 $\mu\text{m/l}$, 0.56 NAA, therefore, no elongation media were necessary. However, Bai et al. (2023) verify the effects of light quality especially of light-emitting diodes (LEDs) on the *in vitro* regeneration of *Lobelia chinensis*. They observed that Red-blue (RB) treatments were suitable for shoot regeneration, shoot growth, and root regeneration from shoots. HPLC analysis of plants revealed the highest accumulation of catechin and myricetin in the plants regenerated under RB light sources. Bai et al. (2024) also describe a micropropagation experiment using nodal segment explants and different growth media (MS, B5, SH, WPM, N6, NLN) without plant growth regulator supplements. They focused on variation of media parameters on plant regeneration, antioxidant activity and secondary metabolite accumulation. They did not do mass propagation and acclimatization experiments for conservation of this plant species. In another experiment Bai et al. (2025) enhance the phenolic and polyacetylene accumulation in *L. chinensis* plantlet cultures through yeast extract and salicylic acid elicitation.

In another set of experiments, different concentrations of Kn (2.78-11.10 $\mu\text{m/l}$) were tested in combination with 0.56 $\mu\text{m/l}$ of NAA, IAA, and IBA. Among the Kn + NAA combinations, the maximum shoot regeneration ($70 \pm 6\%$), highest number of shoots per explant (10.00 ± 0.58), and maximum shoot length (2.00 ± 0.29 cm) were observed in MS medium supplemented with 5.55 $\mu\text{m/l}$ Kn + 0.56 $\mu\text{m/l}$ NAA. Similarly, in the Kn + IAA combinations, the highest shoot regeneration percentage ($63 \pm 3\%$), maximum number of shoots per explant (8.67 ± 0.67), and maximum shoot length (2.00 ± 0.29 cm) were also recorded in 5.55 $\mu\text{m/l}$ Kn + 0.56 $\mu\text{m/l}$ IAA. In the Kn + IBA combinations, the highest shoot regeneration response ($57 \pm 9\%$) and maximum number of shoots per explant (9.00 ± 0.58) were obtained in 5.55 $\mu\text{m/l}$ Kn + 0.56 $\mu\text{m/l}$ IBA, whereas the highest shoot length (2.00 ± 0.29 cm) was recorded in 8.33 $\mu\text{m/l}$ Kn + 0.56 $\mu\text{m/l}$ IBA. The effectiveness of kinetin in promoting shoot regeneration has been reported in previous studies on various plant species (Abu-Romman et al. 2015, Adugna et al. 2020).

For the rooting experiments, full- and half-strength MS media supplemented with different auxins were used. Full-strength MS medium fortified with 5.55 $\mu\text{m/l}$ NAA gave the best response (Table 2). At this concentration, the percentage of root formation was 99.00, the average number of roots increased to 29.33 ± 0.67 within 30 days of transplanting, whereas the average root length (2.83 ± 0.17 cm) was found to be better in MS medium supplemented with 8.33 $\mu\text{m/l}$ NAA (Table 2, Fig. 1E). On the other hand, half-strength MS medium supplemented with different concentrations of auxins also showed responses of varying magnitudes. Here, all the tested concentrations produced roots, but the highest root formation (80%) was recorded in $\frac{1}{2}\text{MS} + 5.55 \mu\text{m/l}$ NAA. The effects of auxins on root formation in *in vitro* developed plants have previously been reported in *Berberis chitria*, *Curcuma longa*, and *Thalictrum foliolosum* (Sharma et al. 2022, Rani et al. 2023, Rani et al. 2024).

Table 2. *In vitro* rooting of *L. chinensis* on half and full strength of MS medium with or without auxins.

Treatments	Con.	Root formation (%)	Shoot/explant (mean ± SE)	Root length (mean ± SE)
Medium	Auxin (µm/l)			
½MS	Control	52 ± 2 ^l	16.66 ± 1.87 ^e	1.06 ± 0.50 ^e
	NAA 2.78	70 ± 0 ^g	19.33 ± 0.67 ^{de}	1.67 ± 0.33 ^{de}
	5.55	80 ± 3 ^{cde}	21.67 ± 1.67 ^{cd}	1.83 ± 0.17 ^{cd}
	8.33	78 ± 2 ^{def}	19.0 ± 1 ^{de}	2.33 ± 0.17 ^{abcd}
	IAA 2.78	70 ± 3 ^g	9.33 ± 0.67 ^f	2.33 ± 0.17 ^{abcd}
	5.55	75 ± 1 ^{efg}	12.33 ± 0.88 ^f	2.67 ± 0.33 ^{ab}
	8.33	70 ± 2 ^g	8.67 ± 0.67 ^f	2.33 ± 0.44 ^{abcd}
	IBA 2.78	70 ± 3 ^g	23.0 ± 1.53 ^{bc}	2.00 ± 0.0 ^{bcd}
	5.55	73 ± 1 ^{fg}	24.0 ± 0.58 ^{bc}	2.33 ± 0.17 ^{abcd}
	8.33	70 ± 0 ^g	8.67 ± 0.88 ^f	2.50 ± 0.29 ^{abc}
MS	NAA 2.78	85 ± 1 ^{bc}	24.0 ± 0.58 ^{bc}	2.50 ± 0.29 ^{abc}
	5.55	99 ± 1 ^a	29.33 ± 0.67 ^a	2.50 ± 0.00 ^{abc}
	8.33	90 ± 1 ^b	25.33 ± 0.33 ^b	2.83 ± 0.17 ^a
	IAA 2.78	80 ± 3 ^{cde}	17.33 ± 1.76 ^e	2.33 ± 0.17 ^{abcd}
	5.55	82 ± 1 ^{cd}	18.0 ± 1.53 ^e	2.17 ± 0.17 ^{abcd}
	8.33	80 ± 1 ^{cde}	17.0 ± 1.53 ^e	2.17 ± 0.17 ^{abcd}
	IBA 2.78	78 ± 1 ^{def}	16.33 ± 1.86 ^e	2.50 ± 0.29 ^{abc}
	5.55	80 ± 0 ^{cde}	19.0 ± 2.08 ^{de}	2.50 ± 0.50 ^{abc}
	8.33	75 ± 1 ^{efg}	12.33 ± 0.33 ^f	2.17 ± 0.17 ^{abcd}

The data are presented as the mean value ± standard error (SE). Mean values followed by the same letters within the same column are not significantly different based on Duncan's multiple range test conducted at a 95% confidence level.

Shoots with well-developed roots were planted in seedling tray containing coco peat. Those were acclimatized for another one week in growth chamber. To prevent evaporation and facilitated light transportation, pots were covered by transparent polythene. After one week seedling tray with survived plants moved to outside the growth room and gradually acclimatized in natural environment (Fig. 1F). Finally the seedlings were transferred to earthen pots where ninety per cent of the plantlets survived in natural conditions without any abnormal morphological changes. These findings substantiate the feasibility and prevision of the developed regeneration protocols for healthy and large-scale *in vitro* propagation of *Lobelia chinensis*.

References

Abu-Romman SM, Al-Hadid KA and Arabiyyat AR (2015) Kinetin Is the Most Effective Cytokinin on Shoot Multiplication from Cucumber. *J. Agricult. Sci.* 7(10): 2015

- Adugna AY, Feyissa T and Tasew FS** (2020) Optimization of growth regulators on *in vitro* propagation of *Moringa stenopetala* from shoot explants. *BMC Biotech.* **20**: 1-10.
- Bai X, Lee HS, Han JE, Murthy HN and Park SY** (2025) Enhancement of Phenolic and Polyacetylene Accumulation in *Lobelia chinensis* (Chinese lobelia) Plantlet Cultures Through Yeast Extract and Salicylic Acid Elicitation. *Horticulturae* **11**: 612.
- Bai X, Lee HS, Murthy H N, Kwon HJ, Yeon SH, Ju JY and Park SY** (2024) Micropropagation of *Lobelia chinensis* Lour.: Influence of medium parameters on plant regeneration, antioxidant activity, and secondary metabolite accumulation. *Korean J. Plant Res.* **37**(3): 225-234.
- Bai XL, Murthy HN and Park SY** (2023) *In vitro* propagation of *Lobelia chinensis* Lour. under different LED lights. *Rom Biotech. Lett.* **28**(2): 3942-3949.
- Beom GJ, Park YH, Kim KH, Kim SN and Yang MH** (2021) Simultaneous Determination of Four Marker Compounds in *Lobelia chinensis* Lour. Extract by HPLC-PDA. *Appl. Sci.* **11**: 12080.
- Biswas A, Bari MA, Roy M and Bhadra SK** (2009) Clonal Propagation Through Nodal Explant Culture of *Boerhaavia diffusa* L. - A Rare Medicinal Plant. *Plant Tiss. Cult. Biotech.* **19**(1): 53-59.
- Biswas A, Bari MA, Roy M and Bhadra SK** (2011) *In vitro* propagation of *Stemona tuberosa* Lour. - a rare medicinal plant through high frequency shoot multiplication using nodal explants. *Plant Tiss. Cult. Biotech.* **21**(2): 151-159.
- Felpin FX and Lebreton J** (2004) History, chemistry and biology of alkaloids from *Lobelia inflata*. *Tetrahedron* **60**(45): 10127-10153.
- Goyal KA, Pradhan S, Basistha CB and Sen A** (2015) Micropropagation and assessment of genetic fidelity of *Dendrocalamus strictus* (Roxb.) nees using RAPD and ISSR markers. *3 Biotech.* **5**: 473-482.
- Islam ABMR and Hossain ABME** (2008) *Lobelia*, In: Ahmed ZU, Hassan MA, Begum ZNT, Khondker M, Kabir SMH, Ahmad M, Ahmed ATA, Rahman AKA and Haque EU (eds.) 2008. *Encyclopedia of Flora and Fauna of Bangladesh*, Vol. 7, *Angiosperms: Dicotyledons (Balsamiaceae-Euphorbiaceae)*. Asiatic Society of Bangladesh, Dhaka, pp. 153-156.
- Jo BG, Park YH, Kim, KH, Kim SN and Yang MH** (2021) Simultaneous Determination of Four Marker Compounds in *Lobelia chinensis* Lour. Extract by HPLC-PDA. *Appl. Sci.* **11**(24): 12080.
- John MK, Gichira AW, Zhao S, Chen JM, Chen LY and Wang QF** (2020) Distribution pattern and habitat preference of *Lobelia* species (Campanulaceae). *PhytoKeys* **159**: 45-60.
- Joshi S, Mishra D, Bisht, G and Khetwal KS** (2011) Essential oil composition and antimicrobial activity of *Lobelia pyramidalis* Wall. *EXCLI J.* **10**: 274-279.
- Komakech R, Kim YG, Kim WJ, Omujal F, Yang S, Moon BC, Okello D, Rahmat E, Kyeyune GN, Matsabisa MG and Kang Y** (2020) A Micropropagation Protocol for the Endangered Medicinal Tree *Prunus africana* (Hook f.) Kalkman: Genetic Fidelity and Physiological Parameter Assessment. *Front. Plant Sci.* **11**: 548003.
- Nahar K, Kashem MA, Bhowmik TK and Rahman MM** (2025) *In vitro* clonal propagation of *Physalis minima* L. - a medicinal herb using nodal explants *Plant. Tiss. Cult. Biotech.* **35**(2): 361-369. DOI: <https://doi.org/10.3329/ptcb.v35i2.86676>
- Paul A and Biswas A** (2024) *In vitro* propagation of *Leucas biflora* (Vahl) Sm. - a vulnerable medicinal herb. *Plant Tiss. Cult. Biotech.* **34**(1): 49-54.

- Rani M, Miah MAH, Hasan MT, Rashid MH, Yasmin S and Haque MS** (2024) Clonal propagation of turmeric (*Curcuma longa*) and confirmation of genetic fidelity of the micropropagated shoots by RAPD markers. *Plant Tiss. Cult. Biotech.* **34**(1): 55-69.
- Rani S, Puri R, Boora P, Qasim A and Mehta M** (2023) Development of an *in vitro* propagation protocol for *Thalictrum foliolosum*: an endangered medicinal plant. *Plant Tiss. Cult. Biotech.* **33**(1): 47-56.
- Reddy MP** (2011) *In vitro* plant propagation: A review. *J. Fore. Sci.* **27**(2): 61-72.
- Sadeghi F, Yadollahi A, Kermani JM and Eftekhari M** (2015) Optimizing culture media for *in vitro* proliferation and rooting of tetra (*Prunus empyrean* 3) rootstock. *J. Genet. Eng. Biotech.* **13**: 19-23.
- Sharma S, Harcharan S. Dhaliwal and Sharma V** (2022) *In vitro* Micropropagation of *Berberis chitria* (Lindl.) - a rare medicinal plant from Himachal Pradesh, India. *Plant Tiss. Cult. Biotech.* **32**(2): 227-236.
- Shibano M, Tsukamoto D, Masuda A, Tanaka Y and Kusano G** (2001) Two new pyrrolidine alkaloids from *Lobelia chinensis* Lour. *Chem. Pharma. Bull.* **49**: 1362-1365.
- Singh RK and Diwakar PC** (2010) The genus *Lobelia* (Lobeliaceae) in India. *Indian J. Fore.* **33**(4): 625-634.
- Sit NW, Chan YS, Chuah BL, Cheng RJ, Leong WM and Khoo KS** (2017) Antiviral, antifungal and antibacterial activities of the chinese medicinal plants, *Houttuynia cordata*, *Lobelia chinensis* and *Selaginella uncinata*. *Southeast Asian J. Trop Med. Pub. Heal.* **48**(3): 616-627.
- Siwach P and Gill RA** (2011) Enhanced shoot multiplication in *Ficus religiosa* L. in the presence of adenine sulphate, glutamine, and phloroglucinol. *Physiol. Mol. Biol. Plants* **17**: 271-280.
- Wang H, Li Y, Huang Y, Zhao C and Cheung HY** (2018) Chemical Profiling of *Lobelia chinensis* with High-Performance Liquid Chromatography/Quadrupole Time-of-Flight Mass Spectrometry (HPLC/Q-TOF MS) Reveals Absence of Lobeline in the Herb. *Molecules* **23**(12): 3258.
- Wiert C** (2006) Medicinal plant of Asia-Pacific-drugs for the future. World Scientific Publishing Co. Pte. Ltd., British Library Cataloguing-in-Publication Data, 380.
- Zhang L, Reddy N, Khoo C, Koyyalamudi SR and Jones CE** (2018) Antioxidant and immunomodulatory activities of polysaccharides isolated from *Lobelia chinensis* Lour. *Pharmacologia* **9**(4): 157-168.

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