Indirect Organogenesis of *Lobelia chinensis*

Weng Hing Thong

Abstract**—***Lobelia chinensis* is an important medicinal herb. It possesses low growth rate and easily got microbial infection. Therefore, the present study was carried out to investigate the indirect organogenesis of *L. chinensis*. Various explants, namely leaves, nodal segments and roots were cultured on MS medium supplemented with different concentrations of 2,4-D. The results showed that 2.0 mg/L 2,4-D induced the highest frequency of callus formation, 79.2%, for leaf explants. After being transferred to organogenesis stimulating medium, leaf explants derived callus exhibited the highest organogenesis frequency, 30.0% and maximum multiple shoots formation, 12.9 shoots. This indirect organogenesis technology would serve as an alternative *in vitro* propagation system for *L. chinensis.*

Keywords**—**Callus, indirect organogenesis, multiple shoots.

I. Introduction

LOBELIA chinensis is a medicinal herb from family Lobeliaceae, commonly used to reduce inflammation, contracts tissues, clears toxins and treats disorders such as fever, jaundice, swelling, and schistosomiasis [1] [2] [3].

Callus production was influenced by the growth regulators supplemental basal media, genotype, the age, and developmental stage of explant, temperature and light [4] [5]. Organs are formed from callus tissues derived from single cells or several cells which divide to give rise to groups of meristemoids [6] [7]. Organogenesis occurs in various plant tissue cultures in response to exogenously added phytohormones, mainly auxin and cytokinin [8]. It has been shown that the indirect shoot organogenesis from callus cultures can be used as an effective method for multiplication of medicinal plants such as *Coleus forskohlii* [9] and *Curcuma longa* [10].

L. chinensis possesses low growth rate and easily infected by pathogens in the field. Propagation via organogenesis is one of the most efficient ways of mass production of plant materials [11]. Therefore, the major objectives of this research were a) to investigate the effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on callus induction and, b) to investigate the effects of 6-benzyladenine (BA) on indirect organogenesis of *L. chinensis*,

II. Materials And Methods

A. Plant Materials

In vitro plantlets of *L. chinensis* were used as source of plant materials.

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B. Effect of 2,4-D on Callus Induction

Different explants, namely leaves 4-8 mm, nodal segments 5-8 mm, and roots 8-10 mm, were used as plant materials for callus induction. One explant was cultured in each vial containing 10.0 mL of Murashige and Skoog medium (1962) (MS) [12] basal medium supplemented with 1.0, 2.0, 3.0, 4.0, and 5.0 mg/L 2,4-D . Thirty samples were used for each treatment and the experiment was repeated three times. MS medium devoid of 2,4-D served as the control. The cultures were incubated at 25±2°C in the dark and were observed daily for one month to determine the time of callus induction and percentage of callus formed.

C. Effect of BA on Indirect Organogenesis

Calli initiated were transferred onto MS medium supplemented with 1.0 and 2.0 mg/L BA to determine the shoot differentiation efficiency of the callus. BA free MS medium served as the control. Ten samples were used for multiple shoot formation and the experiment was repeated twice. All cultures were incubated at 25±2°C under a 16 hours photoperiod provided by cool-white fluorescent lamps and 8 hours of darkness. The percentage of shoot regeneration and the number of shoots produced were recorded.

III. Results And Discussions

A. Effect of 2,4-D on Callus Induction

When the various explants of *L. chinensis* were cultured on MS medium without plant growth regulator, callus was not produced. Similarly, [13] also observed that calli were unable to initiate from neither leaves nor petioles when they were cultured in MS medium devoid of 2,4-D or picloram.

Table 1 showed that for leaf explants, 2.0 mg/L 2,4-D induced the highest frequency of callus formation, 79.2%, followed by 1.0 mg/L 2,4-D which produced 76.6% of callus. The results showed that 1.0 mg/L 2,4-D induced 48.3% and 35.8% of callus formation for nodal segments and roots explants, respectively. When the concentrations of 2,4-D increased, the percentage of callus formation decreased for all the explants. In this study, root explants were less responsive and exhibited low frequency of callus induction. Likewise, leaf and scape of *Eryngium foetidum* were most effective for callus induction, followed by stem and root [14]. The rate of callus regeneration depended on the explants source and plant growth regulators [15]. In many protocols, the callus induction medium contained auxin, usually 2,4-D, which had been widely accepted to be the principal controlling factor to

induce callus formation and growth [4] [16]. Reference [17] also reported that 2,4-D was the most effective auxin for callus induction among the other auxins tested.

TABLE I
EFFECT OF DIFFERENT CONCENTRATIONS OF 2, 4-D ON CALLUS INDUCTION FROM LEAVES, NODAL SEGMENTS AND ROOT EXPLANTS OF *L. CHINENSIS* AFTER FOUR WEEKS OF CULTURE

Plant growth regulators	Percentage of explants forming callus		
	Leaves	Nodal segments	Roots
MS (control)	0.00	0.00	0.00
1.0 mg/L 2,4-D	76.6	48.3	35.8
2.0 mg/L 2,4-D	79.2	34.2	18.3
3.0 mg/L 2,4-D	65.0	31.7	15.0
4.0 mg/L 2,4-D	64.2	31.7	14.2
5.0 mg/L 2,4-D	47.5	27.5	10.0

The callus production of leaves and nodal segments of *L. chinensis* occurred mostly on ninth to tenth day of inoculation in the dark while roots explants took longer time. Callus formation occurred at wounded edge of the explants and gradually covered the whole explant. Similarly, it was reported that callus induction of sorghum was observed as early as 10 days [18]. Similarly, after three weeks of incubation, *Silene vulgaris* leaf explants incubated on MS basal media with different concentrations of auxin/cytokinin demonstrated initial callus formation from the wounded margins of the leaf [5]. Time taken for callus induction, amount, color and texture of callus formation varied between the explant types as well as types, concentrations, and different combinations of plant growth regulators [19] [20].

B. Effect of BA on Indirect Organogenesis

The callus transferred to BA supplemented MS medium gradually became green in color and exhibited nodular structure which had the potential to form adventitious shoot in shoot induction medium. After the callus became green in color, formation of adventitious shoot buds was noticed from the surface of the nodular callus within four to five weeks of culture. Formation of leaves and shoot elongation was noticed within eight weeks of culture. After elongation, the shoots were transferred to hormone free MS medium for rooting. Reference [21] also reported that the calli of *Psoralea corylifolia* differentiated into green nodular structures which developed into dark green shoot buds in the media supplemented with 2.5 mg/L BA and 1.0 mg/L NAA (Naphthaleneacetic acid). In addition, [22] observed that green or green purple compact nodules from calli indicated the initiation of adventitious bud differentiation. Likewise, it was reported that the best embryogenic callus of *Gossypium hirsutum* was light parrot-green in color and featured a grainy, nodular texture [23]. It was noticed that callus transferred to MS medium without BA was not responsive and regenerative.

In this study, it was observed that leaves and nodal segments derived callus of *L. chinensis* were more regenerative than callus developed from roots (Table 2). Root derived callus was not regenerative and exhibited the lowest frequency of shoot regeneration which could be neglected. In this study, for leaf explant derived callus, the highest frequency of shoot regeneration, 30.0%, was induced at 1.0 mg/L BA and it achieved the maximum number of shoots, 12.9 shoots on the eighth week. It was reported that cytokinins induced mostly shoot and root differentiation and elongation [24].

TABLE II
EFFECT OF BA ON THE PERCENTAGE OF SHOOT ORGANOGENESIS AND NUMBER OF SHOOTS PRODUCED FROM CALLUS ON THE EIGHT WEEKS OF CULTURE

Composition of shoot induction medium	Percentage of organogenesis			Means of shoots formed ± S.D.		
	Leaves	Nodal segments	Roots	Leaves	Nodal segments	Roots
MS (control)	0	0	0	0	0	0
MS+1.0 mg/L BA	30.0	34.2	0	12.9±0.3 a	12.8±0.4 b	0
MS+2.0 mg/L BA	23.3	25.0	0	11.8±0.3 b	12.2±0.5 c	0

*Values followed by different letters differ significantly at $P \leq 0.05$ by DMRT.
S.D. reprsents standard deviations of the means.

REFERENCES

[1] H. Tada, K. Shimomura, and K. Ishimaru, "Polyacetylenes in hairy root cultures of *Lobelia chinensis* Lour,," *Journal of Plant Physiology*, vol. 146, pp. 199-202, June 1995.

[2] Z.Q. Lei, *Zhongyaoxue*. China: Shanghai Science and Technology Publisher, 2009, pp. 83-84.

[3] Y. K. Ling, *Zhongyaoxue*, China: Shanghai Science and Technology Publisher, 2008, pp.55.

[4] X. J. Ge, Z. H. Chu, Y. J. Lin, and S. P. Wang, "A tissue culture system for different germplasms of indica rice," *Plant Cell Reports*, vol. 25, pp. 392–402, May 2006.

[5] E. M. Jack, S. Anatasova, and J. A. C. Verkleij, "Callus induction and plant regeneration in the metallophyte *Silene vulgaris* (Caryophyllaceae)," *Plant Cell, Tissue and Organ Culture*, vol. 80, pp. 25–31, Jan. 2005.

[6] J. Muoma, G. Muluvi, and J. Machuka, "*In vitro* regeneration by indirect organogenesis of selected kenyan maize genotypes using shoot apices," *Biotechnology*, vol. 7, no. 4, pp. 732-738, 2008.

[7] N. Selvaraj, A. Vasudevan, M. Manickavasagam, S. Kasthurirengan, and A. Ganapathi, "High frequency shoot regeneration from cotyledon explants of cucumber via organogenesis," *Scientia Horticulturae*, vol. 112, no. 1, pp. 2-8, 2007.

[8] H. Mercier, B. M. Souza, J. E. Kraus, R. M. Hamasaki, and B. Sotta, "Endogenous auxin and cytokinin contents associated with shoot formation in leaves of pineapple cultured *in vitro*," *Brazilian Journal of Plant Physiology*, vol. 15, no. 2, pp. 107-112, May/Aug. 2003.

[9] P. S. Reddy, R. Rodrigues, and R. Rajasekharan, "Shoot organogenesis and mass propagation of *Coleus forskohli* from leaf derived callus," *Plant Cell, Tissue and Organ Culture*, vol. 66, pp. 183-188, Sep. 2001.

[10] N. D. Salvi, L. George, and S. Eapen, " Plant regeneration from leaf base callus of tumeric and random amplified polymorphic DNA analysis of regenerated plants," *Plant Cell, Tissue and Organ Culture*, vol. 66, pp. 113-119, Aug. 2001.

[11] L. Zhang, T. F. Xu, X. F. Sun, and H. M. Zhang, "Factors influencing shoot regeneration from cotyledons of tetraploid *Isatis indigotica* Fort.," *In Vitro Cellular and Developmental Biology: Plant*, vol. 39, no. 5, pp. 459, Sep. 2003.

[12] T. Murashige, and F. Skoog, "A revised medium for rapid growth and bioassay with tobacco tissue culture," *Physiologia Plantarum*, vol. 15, pp. 473-478, July 1962.

[13] C. L. Tan, and L. K. Chan, "Induction of callus from leaves and petioles of *Spilanthes acmella*," in *Future scenario in biological research, insight and co-operation, Proceedings of the Fourth Regional IMT-GT UNINET Conference*, Pulau Pinang, Malaysia, 2002, pp. 206-209.

[14] K. P. Martin, "Efficacy of different growth regulators at different stages of somatic embryogenesis in *Eryngium foetidum* L. - A rare medicinal plant," *In Vitro Cellular and Developmental Biology: Plant*, vol. 40, no. 5, pp. 459463, Sep./Oct. 2004.

[15] D. Li, J. Zhang, J. Zhao, Y. Zhang, F. Chen, J. Zhu, S. Liu, and Z. Yang, "Plant regeneration via somatic embryogenesis of *Elymus sibiricus* cv. 'chuancao no. 2'," *Plant Cell, Tissue and Organ Culture*, vol. 84, no. 3, pp. 285-292, Mar. 2006.

[16] S. K. Sharma, G. J. Bryan, and S. Millam, "Auxin pulse treatment holds the potential to enhance efficiency and practicability of somatic embryogenesis in potato," *Plant Cell Reports* vol. 26, pp. 945–950, July 2007.

[17] B. S. Bhau, andA. K. Wakhlu, "Effect of genotype, explant type and growth regulators on organogenesis in *Morus alba*," *Plant Cell, Tissue and Organ Culture*, vol. 66, pp. 25-29, July 2001.

[18] N. S. Kishore, K. .B.R. S. Visarada, Y. A. Lakshmi, E. Pashupatinath, S. V. Rao, and N. Seetharama, "*In vitro* culture methods in sorghum with shoot tip as the explant material," *Plant Cell Reports*, vol. 25, pp. 174–182, Mar. 2006.

[19] S. Manjkhola, U. Dhar, and M. Joshi, "Organogenesis, embryogenesis, and synthetic seed production in *Arnebia euchroma*–a critically endangered medicinal plant of the Himalaya," *In Vitro Cellular and Developmental Biology: Plant*,vol. 41, no. 3, pp. 244-248, May 2005.

[20] M. R. Beena, and K. P. Martin, "*In vitro* propagation of the rare medicinal plant *Ceropegia candelabrum* L. through somatic embryogenesis," *In Vitro Cellular and Developmental Biology: Plant*, vol. 39, no. 5, pp. 510-513, Sep./Oct. 2003.

[21] C. Saxena, S. K. Palai, S. Samantaray, G. R. Rout, and P. Das, "Plant regeneration from callus cultures of *Psoralea corylifolia* Linn.," *Plant Growth Regulation*, vol. 22, pp. 13–17, June 1997.

[22] D. Xie, and Y. Hong, "*In vitro* regeneration of *Acacia mangium* via organogenesis," *Plant Cell, Tissue and Organ Culture*, vol. 73, pp. 281-284, Sep. 2001.

[23] R. Mishra, H. Y. Wang, N. R. Yadav, and T. A. Wilkins, "Development of a highly regenerable elite Acala cotton (*Gossypium hirsutum* cv. Maxxa) – a step towards genotype-independent regeneration," *Plant Cell, Tissue and Organ Culture*, vol. 73, pp. 21–35, 2003.

[24] M. Guohua, " Effects of cytokinins and auxins on cassava shoot organogenesis and somatic embryogenesis from somatic embryo explants," *Plant Cell, Tissue and Organ Culture*, vol. 54, pp. 1–7, July 1998.