Research Article

Micropropagation and *In Vitro* Inflorescence of *Pentas lanceolata*

Kitti Bodhipadma, Sompoch Noichinda, Chutikarn Tangtivaporn and Saowaros Phanomchai
Division of Agro-Industrial Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand

David W. M. Leung*
School of Biological Sciences, University of Canterbury, Christchurch, New Zealand

* Corresponding author. E-mail: david.leung@canterbury.ac.nz DOI: 10.14416/j.asep.2022.01.004
Received: 11 October 2021; Revised: 22 November 2021; Accepted: 9 December 2021; Published online: 13 January 2022

Abstract
In this study, different concentrations of 6-benzyladenine (BA) on *in vitro* shoot and inflorescence inductions of *P. lanceolata* were investigated. The *in vivo* and *in vitro* floral characteristics of this plant were also compared. Nodal explants of *P. lanceolata* were cultured vertically with the cut ends inserted into semi-solid Murashige and Skoog (MS) medium supplemented with 0, 0.5, 1, 2, 4, and 8 mg L^{-1} BA. The results showed that the explants formed the highest numbers of shoots even when cultured in MS basal medium without any addition of BA, while the shoots formed in the explants cultured in MS medium supplemented with 1 mg L^{-1} BA were the longest. No inflorescence was found in the shoots cultured in MS medium supplemented with 8 mg L^{-1} BA, while the highest percentage of inflorescence induction was found in the shoots cultured in the medium supplemented with 0.5 mg L^{-1} BA. The apperances of *in vivo* and *in vitro* flowers of *P. lanceolata* were the same in many aspects except that the number of flower/inflorescence formed was different. In addition, water accumulation was observed only inside the *in vitro* flowers. Water deposit in the long tubular structure of *P. lanceolata* flower could cause anther injury, suggesting that flowers developed *in vitro* may not always produce pollen.

Keywords: Anther damage, *In vitro* flower, Pollen production

1 Introduction

The beautiful inflorescence of *Pentas lanceolata* (Forssk.) Deflers makes this ornamental plant well-known and widely grown in Thailand. It is thought to originate from tropical East Africa to Arabia and is planted later all over the tropical and subtropical areas. It belongs to the *Rubiaceae* family and contains a terminal inflorescence of umbel-like form. There are many flowers in an inflorescence, which has a narrow cylindrical tube and star-shaped petal with various shades of colors (i.e., red, pale violet, pink, and white, or sometimes of 2 tone-color) [1]–[3].

The inflorescence of *P. lanceolata* is attractive to many insects, including butterflies and bees. A butterfly garden is constructed with *P. lanceolata* for this purpose. In ethnobotany, the plant is principally used against lung diseases and for wound-healing or as an anti-malarial and analgesic drug [4], [5].

Induction of a single *in vitro* flower has been reported in many studies, but there are few studies on inflorescence initiation under aseptic conditions. To induce inflorescence *in vitro*, plant growth regulator might be one of the factors. After 10 months of subculture, inflorescence was initiated in multiple shoots of bamboo from nodal explants cultured in MS medium containing 0.1 mg L^{-1} thidiazuron, [6], while plantlets derived from nodal explants of common cockscomb produced inflorescence when cultured in MS medium supplemented with 0.5 mg L^{-1} 6-benzyladenine (BA) for 5 weeks [7]. In the case of *Dioscorea zingiberensis*, explants cultured in MS medium supplemented with

either kinetin, 1-naphthalene acetic acid, or indole-3-acetic acid could not induce inflorescence formation, but those cultures in medium with BA were able to initiate inflorescence formation. The maximum percentage of inflorescence induction was achieved with a combination of 2 mg L^{-1} BA and 0.5 mg L^{-1} indole-3-butyric acid [8]. Therefore, in the literature, BA appears to be a plant growth regulator that could promote shoot development and inflorescence formation in explants during *in vitro* culture. Since there is no prior published study on successful micropropagation and inflorescence induction in *P. lanceolata* , the objective of the present study was to fill in this knowledge gap, and to investigate if different BA concentrations could induce shoot and inflorescence formation in nodal explants of *P. lanceolata in vitro*. A comparative study of the characteristics of *in vitro* and *in vitro* flowers was also carried out.

2 Materials and Methods

2.1 *Plant material*

Pot plants of *Pentas lanceolata* cv. Lucky Star Deep Pink was obtained from the Chatuchak market, Bangkok, Thailand. It was planted and sold in a pot under uncontrolled open-air conditions. Plant age was around 4–5 months old.

2.2 *Surface sterilization*

Nodal parts (1.5 cm length each) of *P. lanceolata* were excised and cleaned under running tap water for 45 min. Then, they were soaked in 10% (v/v) plant- based detergent [St. Andrews Vegetable and Fruit Washing Liquid containing plant cleansers including fatty alcohol from coconut and palm oil, Lion company (Thailand) Ltd., Thailand] for 30 min and washed again under running tap water for 45 min. After this, the nodal explants were surface sterilized to control bacterial and fungal contaminants with 70% (v/v) ethyl alcohol for 2 min before rinsing with sterile distilled water for 2 min. In the final step, the nodal explants were immersed in a solution containing of 10% (v/v) Clorox and 4 drops of Tween 20 for 5 min before being washed in steriled and distilled water 3 times with 3-min soaking for each time.

2.3 *Micropropagation and in vitro inflorescence investigation*

After surface disinfestation, the nodal explants of *P. lanceolata* were cultured vertically with the cut ends inserted into semi-solid MS medium [9] supplemented with 0, 0.5, 1, 2, 4, and 8 mg L^{-1} BA (Sigma, St. Louis, USA). Then, they were kept in a growth room (16 h of lighting from white fluorescent lamps (21.78 μmol m^{-2} s^{-1}) and 8 h of darkness under 25 ± 2 °C) for 6 weeks to investigate the effect of BA on shoot number and length, and for 2 more weeks to determine inflorescence initiation percentage, the number of inflorescences, and the number of flowers per inflorescence.

The stages of *in vivo* and *in vitro* flowers were classified, and flowers at the full bloom of both *in vivo* and *in vitro* were compared. *In vivo* and *in vitro* flowers were also cut open to examine anther under a stereomicroscope (EMZ-TR, Meiji Techno Co., Ltd.). If pollen was present, their sizes were observed under a light microscope (ML2000, Meiji Techno Co., Ltd.) before and after staining with 0.5% (w/v) acetocarmine. The percentage of pollen viability was also determined after counting the number of the red stained pollen and the non-viable pollen which was unstained.

2.4 *Statistical analysis*

Means of shoot number and length, inflorescence number/jar, flower/inflorescence number were compared using Duncan's Multiple Range Test ($p < 0.05$). Means of flower length, petal, stamen, carpel number, flower number/inflorescence, and pollen size were compared using Independent Sample t-Test ($p < 0.05$).

3 Results and Discussion

3.1 *Nodal explant culture of Pentas lanceolata*

Pentas lanceolata flowers all year round, but the best flower quality is found in summer. It has been recommended to propagate *P. lanceolata* vegetatively via the terminal stem cuttings [10]. In this research, vegetative propagation of this ornamental plant species was attempted using nodal segments cultured *in vitro*. Surface sterilization is one of the problems on starting plant tissue culture from pot plants grown in commercial sites, which may generally harbor many

microorganisms from the air or growing media. In our preliminary test, it was found that surface sterilization with 70% ethyl alcohol was a necessary step to eliminate microorganisms, such as bacteria and fungi, from nodal parts of *P. lanceolata*. Without this step, all the explants were contaminated. However, including ethyl alcohol in surface disinfestation resulted in about 83.33% contamination-free nodal explants of *P. lanceolata*.

All *P. lanceolata* nodal explants were able to form shoots when placed vertically with their basal parts inserted into MS agar medium supplemented with various concentrations of BA for 6 weeks (Table 1). This observation suggested that the isolation of the nodal explant from the apical shoot dominance effect was enough for the development of axillary shoot buds in the nodal explants without an exogenous BA in the medium. A possible explanation for this is that removal of the terminal shoot apical tip is known to promote cytokinin content in the axillary buds of the nodes for shoot development [11] and the sugar supplied in the culture medium is also critical to trigger axillary shoot induction from the nodal explants [12]. The nodal explants of many plants from *Rubiaceae* responded very well to BA in the medium for shoot induction. A higher number of shoots per nodal explant of *Morinda officinalis* and *Gynochthodes umbellata* was observed after culturing in MS medium supplemented with BA compared to other cytokinins, e.g., kinetin and thidiazuron [13], [14]. Therefore, it is possible that the requirements for exogenous cytokinin or BA to induce shoot development from the nodal explants may be dependent on the plants studied. For *P. lanceolata*, the number of shoot/jar was not significantly different in MS basal medium without any BA or with up to 4 mg L^{-1} BA (Table 1). This finding was different from those in *M. officinalis* and *G. umbellata*. The number of shoot per jar was significantly different when the explants were cultured on medium supplemented with 8 mg L^{-1} BA than with 0 mg L^{-1} BA. Interestingly, *P. lanceolata* nodal explants appeared to exhibit the longest shoot length per jar when cultured in MS medium supplemented with 1 mg L^{-1} BA although there was no significant difference among those cultured in medium from 0 and up to 4 mg L^{-1} BA (Table 1). The shoot length was significantly lower when cultured in medium supplemented with 8 mg L^{-1} BA than with 1 mg L^{-1} BA (Table 1). In conclusion, exogenous BA is not needed for shoot development from *P. lanceolata* nodal explants.

Table 1: Shoot induction percentage, the average number and length of shoot/jar in nodal explants of *Pentas lanceolata* cultured in semi-solid MS medium supplemented with different concentrations of BA for 6 weeks

MS+BA (mg L^{-1})	% Shoot Induction	No. of Shoot/ jar ± S.E.	Length of Shoot/ jar ± S.E. (cm)
0	100	2.00 ± 0.00 b	2.42 ± 0.20 ab
0.5	100	1.83 ± 0.17 ab	3.42 ± 1.03 ab
1	100	1.50 ± 0.22 ab	5.00 ± 1.35 b
2	100	1.83 ± 0.17 ab	3.58 ± 1.24 ab
4	100	1.83 ± 0.17 ab	2.25 ± 0.73 ab
8	100	1.30 ± 0.21 a	0.75 ± 0.11 a

Data are mean ± SE from 6 replications (1 nodal explant in each jar and there were 6 jars in total), and the means within the same column with different letters are significantly different ($p < 0.05$). % of shoot induction = number of jars with emerging shoots divided by 6 jars x 100%.

3.2 *In vitro inflorescence of Pentas lanceolata*

In vitro inflorescence in *Rubiaceae* was rarely reported. Shoot culture of *Ixora coccinea* formed inflorescence after culturing on woody plant medium (WPM) supplemented with 0.56 mg L^{-1} BA for 8 weeks, while in *Oldenlandia umbellate* a medium supplemented with 1.5 mg L^{-1} BA, 0.7 mg L^{-1} NAA and 0.4% coconut water was required to induce the maximum number of *in vitro* inflorescence [15], [16]. In this research, a new shoot grown from the axillary bud of *P. lanceolata* nodal explants on MS medium supplemented with 0.5 mg L^{-1} BA exhibited floral bud development after around 6 weeks of culturing, while no floral bud was found in the shoots cultured in media supplemented with other BA concentrations (data not shown). After 8 weeks of culture, no inflorescence was found in MS medium supplemented with 8 mg L^{-1} BA, whereas the shoots cultured in the medium supplemented with 0.5 mg L^{-1} BA exhibited the highest percentage of inflorescence induction (Table 2 and Figure 1). There was no significant difference in the number of inflorescence per jar and the number of flower per inflorescence when the explants were cultured in various concentrations of BA (Table 2). Thus, for *P. lanceolata*, a low concentration of BA promoted *in vitro* inflorescence formation, while a high BA concentration inhibited

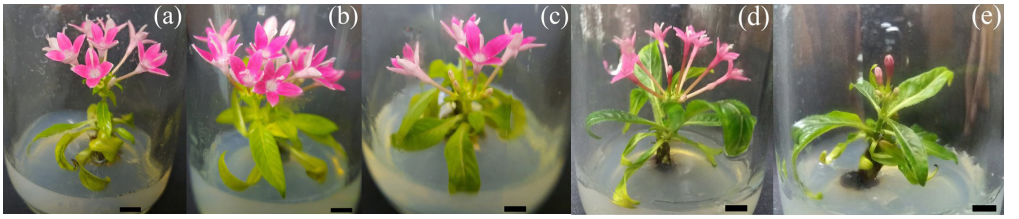

Figure 1: Inflorescence formation on new shoots emerging from nodal explants cultured in semi-solid MS medium supplemented with 0, 0.5, 1, 2, and 4 mg L^{-1} BA (a to e, respectively) for 8 weeks. Scale bar = 0.5 cm.

floral formation *in vitro*. This result was similar to that from studies on explants of *Dianthus chinensis* cultivars, which formed more flowers on a medium supplemented with 0.56 mg L^{-1} BA than that supplemented with 1.13 mg L^{-1} BA [17].

Table 2: Inflorescence induction percentage, the average number of inflorescence/jar and average number of flower/inflorescence after nodal explants of *Pentas lanceolata* were cultured in semi-solid MS medium supplemented with different concentrations of BA for 8 weeks

MS+BA (mg L^{-1})	% Inflorescence Induction	No. of Inflorescence/ jar ± S.E.	No. of Flower/ Inflorescence ± S.E.
0	16.67	0.17 ± 0.17 ab	1.33 ± 1.33 ab
0.5	50	1.00 ± 0.45 b	5.67 ± 2.74 b
1	16.67	0.17 ± 0.17 ab	1.17 ± 1.17 ab
2	16.67	0.17 ± 0.17 ab	1.50 ± 1.50 ab
4	16.67	0.17 ± 0.17 ab	0.67 ± 0.67 a
8	0	0.00 ± 0.00 a	0.00 ± 0.00 a

Data are mean ± SE from 6 replications (1 nodal explant in each jar and there were 6 jars in total), and the means within the same column with different letters are significantly different ($p < 0.05$). % of inflorescence induction = numbers of jars with emerging inflorescence divided by 6 jars x 100%.

During inflorescence development, the *in vivo* and *in vitro* flowers of *P. lanceolata* were compared and classified into 5 stages, as shown in Figure 2. The results revealed that all the characteristics studied such as floral length at the full bloom, numbers of petals, stamen, and carpel of both sources were similar except the number of flower/inflorescence (Table 3). The similarity of *in vivo* and *in vitro* flowers in an inflorescence might depend on the species or family. In common cockscomb and feathered amaranth (Amaranthaceae), florets from both sources largely contained the same numbers and characters of floral parts, except the size

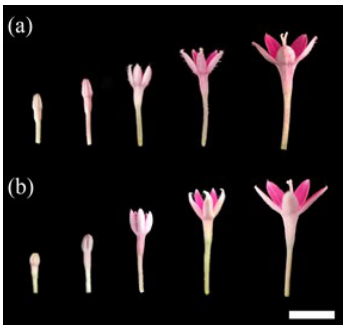

Figure 2: Five developmental stages of *in vivo* (a) and *in vitro* (b) flowers of *Pentas lanceolata*. From left to right were stages 1 to 5, respectively. Scale bar = 1 cm. The *in vitro* flowers of different stages were collected randomly from the shoots formed *in vitro* in semi-solid MS medium without exogenous BA for 8 weeks and the *in vivo* flowers were collected randomly from pot plants grown outdoors.

[7], [18]. However, in *P. lanceolata* and *O. umbellate* (*Rubiaceae*), all floral characters of *in vivo* and *in vitro* flowers, even the size, were the same [16]. In conclusion, the best condition for inflorescence induction from shoots of *P. lanceolata* nodal explants, is the culturing of the explants in MS medium supplemented with 0.5 mg L^{-1} BA.

Table 3: Flower characters of *in vivo* and *in vitro* flower of *Pentas lanceolata*

Flower Characters	*In vivo* Flower	*In vitro* Flower
Length at the full bloom (mm) [X]	19.70 ± 0.12 a	19.64 ± 0.13 a
No. of petal [X]	5.00 ± 0.00 a	5.00 ± 0.00 a
No. of stamen [X]	5.00 ± 0.00 a	5.00 ± 0.00 a
No. of carpel [X]	1.00 ± 0.00 a	1.00 ± 0.00 a
No. of flower/inflorescence [Y]	20.30 ± 1.25 a	6.20 ± 0.65 b

Note: [X] = Data are mean ± SE from 50 flowers and, [Y] = Data are mean±SE from 10 flowers. Means within the same row with different letters are significantly different ($p < 0.05$).

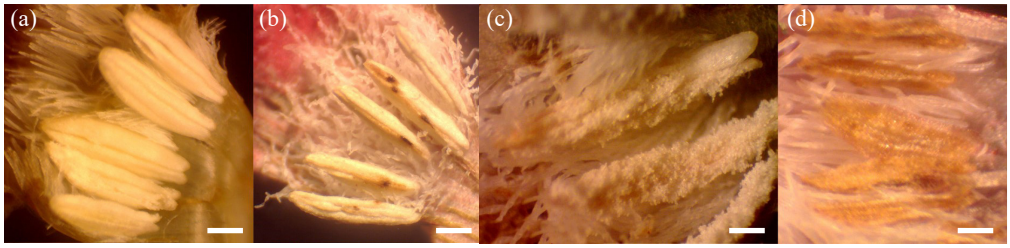

Figure 3: Characteristic of *Pentas lanceolata* anther: (a) and (b) from *in vivo* and *in vitro* flowers, respectively, at stage 2 (Scale bar = 1 mm), (c) and (d) from *in vivo* and *in vitro* flowers, respectively, at stage 3. Scale bar = 0.5 mm. The anthers were from *in vitro* flowers of different stages collected randomly from the shoots formed *in vitro* in semi-solid MS medium without exogenous BA for 8 weeks and the *in vivo* flowers were collected randomly from pot plants grown outdoors.

3.3 *In vivo and in vitro pollen of Pentas lanceolata*

In vitro pollen in *Rubiaceae* has also never been investigated. In this experiment, we tried to observe both *in vivo* and *in vitro* pollen. After further investigation into the inside of the *in vivo* and *in vitro* flowers of *P. lanceolata*, the *in vivo* anthers looked normal and could release pollen at stage 3 flower [Figure 3(a) and (c)], while atrophied and necrotic anthers were found in the *in vitro* flowers at stage 2 [Figure 3(b)], and these *in vitro* anthers turned brown at stage 3 flower [Figure 3(d)]. Moreover, water was also found only inside the long tubular structure of the *in vitro* flower after being cut open. The accumulation of water inside the *in vitro* flower might occur from the very high humidity in the container during culture. Then, this wet environment caused anther atrophy, necrosis, and death, and no pollen was obtained under this condition. Under the controlled condition, *Cistus incanus* and *Myrtus communis* pollen rapidly lost their viability at high humidity [19]. This result suggested that high humidity affected some plants' pollen viability, but in the present research, very high humidity was correlated with anther viability *in vitro*. Thus, only *in vivo* pollen of *P. lanceolata* could be examined further.

The *in vivo* pollen of *P. lanceolata*, before staining, could be classified into 2 groups, large and small, according to the pollen size under a compound light microscope [Table 4 and Figure 4(a)]. After being stained with 0.5% acetocarmine, the pollen viability percentage was around 75.71 (data from 30 replications) and stained red, while the non-viable pollen was unstained [Figure 4(b)]. The shape of viable pollen

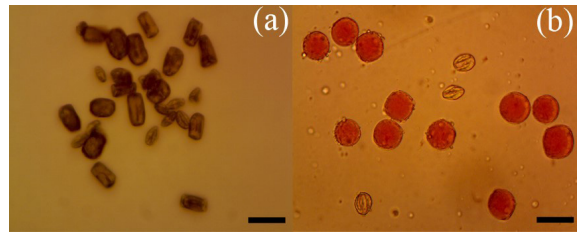

Figure 4: Pollens of *in vivo Pentas lanceolata* flowers: (a) before and (b) after staining. Scale bar = 20 µm.

changed from rectangle to round one, while the non-viable pollen was oval [Table 5 and Figure 4(b)]. The fertility of *P. lanceolata* pollen *in vivo* was nearly similar to that of *Ixora coccinea* cv. 'Dwarf Red Coccinea' (72.05%), though the unstained pollen shape was dissimilar to that of this *Rubiaceae* plant [20].

Table 4: *Pentas lanceolata* pollen size from *in vivo* flower before staining with 0.5% (w/v) acetocarmine

Type of Pollen	Width (µm)	Length (µm)
Large size	13.50 ± 0.15 a	24.79 ± 0.15 a
Small size	8.21 ± 0.15 b	16.06 ± 0.20 b

Note: Data are mean±SE from 100 pollen, and means within the same column with different letters are significantly different ($p < 0.05$).

Table 5: *Pentas lanceolata* pollen size of *in vivo* flowers after staining with 0.5% (w/v) acetocarmine

Type of pollen		
Viable Pollen	Non-viable Pollen	
Diameter (µm)	Width (µm)	Length (µm)
25.77 ± 0.17	12.04 ± 0.16	19.22 ± 0.26

Note: Data are mean ± SE from 100 pollen.

Based on the present research, it seems possible

that the *in vivo* and *in vitro* pollen in *Rubiaceae* has never been compared due to the unavailability of *in vitro* pollen in this family. Although *in vitro* flowers of many plant families could produce flowers or inflorescence *in vitro*, it may be possible that these may not always produce pollen. Nevertheless, it is important to note that for *P. lanceolata*, it was the lifespan of the *in vitro* inflorescence was prolonged for more than 30 days (some could persist until 60 days), whereas that of the *in vivo* inflorescence bloomed on the plant for only around 7–14 days. This observation might be of practical value, for example, *in vitro* inflorescence of *P. lanceolata* as a test-tube gift or as a novel product in the bouquet industry [18].

4 Conclusions

Though micropropagation of *Pentas lanceolata* nodal explant might not require BA, the length of the regenerated shoot seemed to be enhanced in the presence of added BA. Besides, it also affected the inflorescence induction percentage, the average number of inflorescence/jar, and the average number of flower/inflorescence of *P. lanceolata*. The prolonged lifespan of the beautiful inflorescence blooming *in vitro* of *P. lanceolata* may be worthly for further application as well. This might lead to a novel ornamental gift product from this plant biotechnology research.

Acknowledgments

We would like to thank Thanyalak niyomsakul, Songnapa Noiseaweeprasert and Krissana Puntapummee for their kind help in taking some photos for this paper.

References

[1] E. F. Gilman and S. Shiffit, "*Pentas lanceolata* Pentas," Institute of Food and Agricultural Sciences, University of Florida, Florida, USA, FPS-465, 1999.

[2] C. Puff and V. Chamchumroon, "Non-indigenous *Rubiaceae* grown in Thailand," *Thai Forest Bulletin (Botany)*, vol. 31, pp. 75–94, 2003.

[3] J. Schripsema, G. P. Caprini, R. van der Heijden, R. Bino, R. de Vos, and D. Dagnino, "Iridoids from *Pentas lanceolata*," *Journal of Natural Products*, vol. 70, pp. 1495–1498, 2007, doi: 10.1021/np070116.

[4] D. Suman, Y. Vishwanadham, T. Kumaraswamy, P. Shirisha, and K. Hemalatha, "Phytochemical evaluation and analgesic activity of *Pentas lanceolata* leaves," *Natural Products Chemistry & Research*, vol. 2, pp. 135, 2014, doi: 10.4172/2329-6836.1000135.

[5] A. Venditti, L. Guarcini, M. Ballero, and A. Bianco, "Iridoid glucosides from *Pentas lanceolata* (Forssk.) Deflers growing on the Island of Sardinia," *Plant Systematics and Evolution*, vol. 301, pp. 685–690, 2015, doi: 10.1007/s00606-014-1106-9.

[6] C.-S. Lin, C.-T. Chen, C.-C. Lin, and W.-C. Chang, "A method for inflorescence proliferation," *Plant Cell Reports*, vol. 21, pp. 838–843, 2003, doi: 10.1007/s00299-003-0571-3.

[7] K. Bodhipadma, S. Noichinda, I. Yadbuntung, W. Buaeiam, and D.W.M. Leung, "Comparison of *in vitro* and *in vivo* inflorescence of common cockscomb (*Celosia argentea* var cristata)," *ScienceAsia*, vol. 36, pp. 68–71, 2010, doi: 10.2306/ scienceasia1513-1874.2010.36.068.

[8] X.-L. Huang, B. Yang, C.-G. Hu, and J.-L. Yao, "*In vitro* induction of inflorescence in *Dioscorea zingiberensis*," *Plant Cell, Tissue and Organ Culture*, vol. 99, pp. 209–215, 2009, doi: 10.1007/s11240-009-9595-x.

[9] T. Murashige and F. Skoog, "A revised medium for rapid growth and bioassays with tobacco tissue culture," *Physiologia Plantarum*, vol. 15, pp. 473–497, 1962.

[10] W. Horn, "New types of *Pentas lanceolata* from seedling progenies," *Acta Horticulturae*, vol. 252, pp. 105–110, 1989.

[11] S. Shimizu-Sato, M. Tanaka, and H. Mori, "Auxin–cytokinin interactions in the control of shoot branching," *Plant Molecular Biology*, vol. 69, pp. 429–435, 2009, doi: 10.1007/s11103-008-9416-3.

[12] M. G. Mason, J. J. Ross, B. A. Babst, B. N. Wienclaw, and C. A. Beveridge, "Sugar demand, not auxin, is the initial regulator of apical dominance," *Proceedings of National Academy of Sciences of the United States of America*, vol. 111, pp. 6092–6097, 2014, doi: 10.1073/pnas.1322045111.

[13] Z. C. Deng, H. Jin, and H. He, "An efficient

micropropagation system for *Morinda officinalis* How. (*Rubiaceae*), an endangered medicinal plant," *Journal of Agricultural Science and Technology*, vol. 17, pp. 1609–1618, 2015.

[14] S. Anjusha and A. Gangaprasad, "*In vitro* propagation and anthraquinone quantification in *Gynochthodes umbellata* (L.) Razafim. & B. Bremer (Rubiaceae)—A dye yielding plant," *Industrial Crops and Products*, vol. 81, pp. 83–90, 2016, doi: 10.1016/j.indcrop. 2015.11.043.

[15] P. Lakshmanan, C. L. Lee, and C. J. Goh, "An efficient *in vitro* method for mass propagation of a woody ornamental *Ixora coccinea* L.," *Plant Cell Reports*, vol. 16, pp. 572–577, 1997, doi: 10.1007/BF01142326.

[16] S. K. Behera, C. Rajasekaran, S. Payas, D. P. Fulzele, C. G. P. Doss, and R. Siva,"*In vitro* flowering in *Oldenlandia umbellata* L.," *Journal of Ayurveda and Integrative Medicine*, vol. 9, pp. 99–103, 2018, doi : 10.1016/ j.jaim.2017.02.011.

[17] R. Sreelekshmi and E. A. Siril, "Effect of BA on high-frequency *in vitro* flowering in *Dianthus chinensis* L. cultivars- a tool to early screening of variant types," *International Journal of Research and Analytical Reviews*, vol. 6, pp. 10–20, 2019.

[18] K. Bodhipadma, S. Noichinda, C. Yompakdee, W. Tamtimdee, U. Chikhuntod, and D. W. M. Leung, "Comparative study of *in vitro* and *in vivo* inflorescence of feathered amaranth," *KMUTNB International Journal of Applied Science and Technology*, vol. 8, no. 3, pp. 173–177, 2015, doi: 10.14416/j.ijast.2015.06. 001.

[19] G. Aronne, "Effects of relative humidity and temperature stress on pollen viability of *Cistus incanus* and *Myrtus communis*," *Grana*, vol. 38, pp. 364–367, 1999, doi: 10.1080/00173130050136154.

[20] S. Phanomchai, K. Bodhipadma, S. Noichinda, L. Punnakanta, and D. W. M. Leung, "Effect of different pollen harvesting times on quantity, viability and *in vitro* germinability of *Ixora coccinea* 'Dwarf Red Coccinea' pollen," *Biotropia*, vol. 28, pp. 84–91, 2021, doi: 10.11598/btb.0.0.0.1159.