

Some Morphological and Physiological Characters of *Vanda bensonii* Bateman Seedlings under Colchicine Application

Furidian Khairunissa, Murni Dwiati, Agus Hery Susanto*

Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto 53122, Central Java, Indonesia

*Corresponding author email: susanto1408@unsoed.ac.id

Received August 02, 2025; Accepted September 27, 2025; Available online November 20, 2025

ABSTRACT. *Vanda bensonii* is an orchid species of some potentials to develop, one of which is by means of induced mutation resulting in polyploid individuals. Such individuals usually exhibit better phenotypical performance than that of the normal diploids. This can be performed by the application of antimitotic chemicals such as colchicine. This study aims to know the effect of colchicine on some morphological and physiological characters of *V. bensonii* seedlings and to find out the effective concentration in producing the most promising ideal phenotypical performance of the orchid species. The study was conducted at the Plant Physiology Laboratory, the Faculty of Biology, Universitas Jenderal Soedirman by using an experimental method arranged in a Completely Randomized Design with treatments consisting of five colchicine concentrations, i.e. 0, 20, 40, 60, and 80 μM . Each treatment was subjected to five replications giving rise to a total of 25 experimental units. The data obtained were analyzed using ANOVA with an F test of 0.05 and 0.01. Further analysis using Tukey test of 0.05 was performed when significant differences among treatments were found. The results show that colchicine significantly reduces plant height of *V. bensonii*, in which control plant shows taller in comparison to all treatments. Similarly, it is also the case with chlorophyll a content, while there is no significant effect of colchicine on leaf width, leaf length, leaf number, shoot number, chlorophyll b and total chlorophyll contents. Nevertheless, colchicine concentration of 60 μM is found most effective in increasing shoot number. It can be concluded that colchicine has significant effects on chlorophyll a content of *V. bensonii*, implying its potential application to create mutants of better performance.

Keywords: colchicine, morphological characters, physiological characters, polyploid, *Vanda bensonii*

INTRODUCTION

Orchids are mostly known as ornamental plants of relatively high economic value, particularly due to their beauty and uniqueness of flower colour and shape. One species of orchid that has interesting flowers is *Vanda bensonii*, which is native to Indian subcontinent, Thailand and Myanmar. This monopodial epiphytic orchid species has fragrant and durable flowers of superb rust brown colour with a complementary lavender lip (Kirankumar, et al., 2021). *V. bensonii* is also found in the Philippines, which some microalgal communities are associated with its velamen aerial roots (Arguelles, 2025). To increase the phenotypic performance of *V. bensonii*, induced mutation resulting in polyploid individuals can be employed. Such individuals usually exhibit better phenotypical performance in comparison to their normal diploid counterparts. This can be performed by the application of antimitotic chemicals such as colchicine.

Colchicine, a plant water-soluble alkaloid, is often used in plant breeding to create genetic variation. The IUPAC name for this substance is N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-6,7-dihydro-5H-benzo[a]heptalen-7-yl]acetamide with molecular formula of

$\text{C}_{22}\text{H}_{25}\text{NO}_6$ (Dhyani et al., 2022). In the mitotic phase, colchicine prevents the formation of spindle threads. This prevents chromosomes from being drawn to each pole of the cell, which prevents cell division. Therefore, there is an increase in the number of chromosomes in a plant cell, which is called polyploid. The colchicine mechanism of action is inhibiting the formation of microtubules. Colchicine will bind to the tubulin dimer alpha and beta, so that no protofilament will be formed. If the protofilament is not formed, it will not form singlet and duplet microtubules, resulting in no cleavage spindle formation. When the formation of the cleavage spindle is inhibited, the chromosomes that are already in the state of doubling are not separated to the opposite directions (Dwiati & Susanto, 2025).

Induced mutation employing colchicine in another species of orchid, i.e. *Paphiopedilum villosum*, has been reported. *P. villosum* shoots were used as materials for inducing autopolyploidization with colchicine solutions at concentrations of 2, 10, and 50 μM . The treatments were incubated for 3, 6, and 9 days in the dark. It was shown that polyploid induction rate of 19.88% was achieved at colchicine of 50 M. Among the obtained polyploid plantlets, tetraploids

were found the highest, i.e. 88.24%. Colchicine application is proven as an effective method for producing polyploid plantlets for further breeding purposes of *P. villosum* as well as other endangered orchids (Huy et al., 2019). Similarly, colchicine has been successfully applied to induce polyploid in *Dendrobium* 'Transient White Rika' and 'Florenza', resulting in some mutants of potentially better characteristics (Sukanto et al., 2025). Nevertheless, no study on colchicine-induced polyploidy in *Vanda bensonii* has been reported. Hence, this study aims to know the effect of colchicine on some morphological and physiological characters of *V. bensonii* seedlings and to find out the effective concentration in producing the most promising ideal phenotypical performance of the orchid species.

EXPERIMENTAL SECTION

The study was conducted experimentally at the Plant Physiology Laboratory, Faculty of Biology, Universitas Jenderal Soedirman, Indonesia. *V. bensonii* seedlings resulting from an *in vitro* culture were used as the plant materials.

Experimental Design

A Completely Randomized Design (CRD) with treatments comprising five colchicine levels of concentrations, i.e. 0, 20, 40, 60, 80 μM , were employed. Each treatment was subjected to five replications giving rise to a total of 25 experimental units. The parameters to examine were both morphological and physiological characters. The morphological parameters comprised plant height, leaf number, leaf length, leaf width, shoot number, while the physiological parameters consisted of total chlorophyll, chlorophyll a and b contents.

Procedures

Preparation of colchicine stock solution

The colchicine stock solution was made in a total volume of 250 mL at a concentration of 1,000 μM , after which DMSO was added up to 2%. This was obtained by weighing colchicine of 2.4 g and dissolved it with DMSO and aquadest up to a volume of 250 mL.

Preparation of Murashige & Skoog media

There were four different types of Murashige & Skoog (MS) media to be prepared. They were MS liquid media with colchicine addition, MS liquid media without plant growth regulator (PGR) per 1 L, MS solid media without PGR per 1 L and MS solid media with PGR per 2 L.

Culturing explants on colchicine media

V. bensonii explants of 4 months were planted in 10 Erlenmeyers previously filled with colchicine solution and liquid MS media. Then the Erlenmeyers were closed using aluminum foil after which shaking at 100 rpm was performed for 7 days. After 7 days the explants were rinsed three times using sterile aquadest to remove the colchicine. Then the explants were

transferred into new MS liquid media for 7 day-reshaking to enable adaptation of the explant before being deposited into solid media. After 7 days the explants were transferred into the solid MS media and cultured for 1 month.

Subculturing explants to PGR media

After being cultured for 1 month in solid MS media without PGR, then the pH was adjusted to 5.83 by adding 1N NaOH or 1 N HCl as necessary. After pH test, subculturing in PGR media containing 5% coconut water, 3 ppm 6-benzylaminopurine (BAP), 1 ppm kinetin was performed for 2 months.

Subculturing explants to non PGR media

Subculturing was carried out once a month periodically. Subculturing is one of the stages in plant propagation by replacing *in vitro* culture planting media with new media, so that the nutritional needs in plant growth can be fulfilled. The subculture method begins with preparation of media. Subculturing is carried out when the media supply in the bottle has been used up (Guo & Jeong, 2021).

Parameter measurement

During 8 wap (weeks after planting), both morphological and physiological parameters were measured. Samples of leaves were weighed as much as 0.02 g, crushed and dissolved using 2 mL acetone solution and filtered. This was repeated several times until the leaf extract was not green any longer. Then, the extract was put into a test tube for measurement in a spectrophotometer of 646 nm and 663 nm wavelengths.

To calculate chlorophyll contents, the following formulas (Chazaux et al., 2022) were employed.

$$\text{Chlorophyll a} = (12.21 \times A_{663}) - (2.81 \times A_{646})$$

$$\text{Chlorophyll b} = (20.13 \times A_{646}) - (5.03 \times A_{663})$$

$$\text{Total chlorophyll} = (17.3 \times A_{646}) - (7.18 \times A_{663})$$

Data Analysis

The data obtained were analyzed using ANOVA with an F test of 0.05 and 0.01. Further analysis using Turkey test of 0.05 was performed when significant differences among treatments were observed.

RESULTS AND DISCUSSION

The effects of colchicine on *V. bensonii* plant height are presented in **Table 1**. The highest plant height is found at 0% concentration (control), while all the treatments show lower plant height despite no statistically significant difference among them is observed. This is also the case with leaf length, even no significant difference from the control is obtained. No significant difference on the morphological parameters other than plant height is observed.

Higher concentrations of colchicine result in shorter *V. bensonii*, mainly in comparison to the control. This is due to the inhibition of cell division. On the other hands, plant cells will undergo multiplication of organelles and chromosomes leading to polyploidy (Mohammadi et al., 2023).

Despite no significant difference, the number of shoots formed indicates that colchicine has a potential for genetic improvement of *V. bensonii*. In comparison to that in control, the shoot number increases approximately 170% at 60 μM concentration of colchicine, although it decreases about half at 80 μM concentration. Colchicine serves as cytokinin growth regulators capable of multiplying shoots and also functions as thidiazuron that is able to induce faster shoot propagation (Kumar, 2024). This is because thidiazuron can stimulate changes in cytokinins to be more active. It seems likely that the higher the concentration of colchicine, the more photosynthates are produced. Hexoses can be used to promote the growth of adventitious shoots, which will be able to serve as the new reproductive tools for the monopodial *V. bensonii* (Li et al., 2022).

With respect to plant height, this study does not correspond to Manzoor et al. (2018) that the decrease in plant height of polyploid plants is probably due to physio-chemical disturbance of cells and induction of polyploidy. The increased plant growth due to polyploidization has also been reported by previous studies. A two-fold increase in stem dry weight of *Arabidopsis* polyploid mutants in comparison to the wild type was obtained (Corneillie et al., 2019). The induced polyploid medicinal orchid (*Anoectochilus formosanus*) was also reported significantly enhanced on various growth parameters including dry weight, fresh weight, shoot length, root length, and leaf width.

The tetraploid had significant higher responses in growth performances, including dry weight, fresh weight, shoot length, root length, and leaf width, when compared with the diploid. Generally, the size of the tetraploid was obviously larger than the diploid. The diploid had a significant higher length and width of the leaf than the tetraploid. Consequently, the leaf shape was dramatically changed by the ploidy level, and the tetraploid had cordate leaves rather than ovate leaves as the typical diploid (Chung et al., 2017).

The results on physiological parameters, including chlorophyll a, chlorophyll b, and total chlorophyll contents, are presented in Table 2. The highest chlorophyll a content is found at 60 μM concentration of colchicine, while the lowest one is observed at 20 μM concentration. The chlorophyll b content shows the highest value at 40 μM concentration of colchicine and the lowest one at 20 μM concentration, while the total chlorophyll content shows the highest value at 60 μM and the lowest one at 20 μM . However, colchicine has only significant effect on chlorophyll a content.

The results on chlorophyll a indicates that the amount of chlorophyll a in *V. bensonii* could be increased due to colchicine treatment at a concentration of 60 μM . Leaves undergo increasing content of chlorophyll a, which is the center of the photosystem I reaction, and serves also as an antenna that will be more effective in capturing light energy.

Table 1. The averages of morphological parameters of *V. bensonii* under colchicine application

Colchicine (μM)	Plant height (mm)	Leaf number	Leaf length (mm)	Leaf width (mm)	Shoot number
0	26.50 $\pm$ 5.16 ^b	5.5 $\pm$ 0.93 ^a	21.4 $\pm$ 5.90 ^a	3.7 $\pm$ 0.76 ^a	0.00 $\pm$ 0.00 ^a
20	17.00 $\pm$ 4.94 ^a	5.1 $\pm$ 1.14 ^a	12.80 $\pm$ 3.46 ^a	4.0 $\pm$ 0.80 ^a	0.9 $\pm$ 0.55 ^a
40	23.00 $\pm$ 3.60 ^a	6.5 $\pm$ 2.32 ^a	18.90 $\pm$ 3.89 ^a	3.8 $\pm$ 1.15 ^a	0.3 $\pm$ 0.45 ^a
60	19.00 $\pm$ 3.18 ^a	7.6 $\pm$ 2.90 ^a	15.00 $\pm$ 3.69 ^a	4.3 $\pm$ 0.44 ^a	1.7 $\pm$ 2.46 ^a
80	19.00 $\pm$ 5.73 ^a	6.0 $\pm$ 0.71 ^a	14.9 $\pm$ 5.28 ^a	4.0 $\pm$ 1.10 ^a	1.1 $\pm$ 0.22 ^a

Note: Numbers followed by the same letter in the same column are not significantly different at the $P < 0.05$ level of significance.

Table 2. The averages of physiological parameters of *V. bensonii* under colchicine application

Colchicine Concentration (μM)	Chlorophyll a ($\mu\text{g/mL}$)	Chlorophyll b ($\mu\text{g/mL}$)	Total chlorophyll ($\mu\text{g/mL}$)
0	2.73 $\pm$ 0.56 ^a	3.03 $\pm$ 0.77 ^a	1.66 $\pm$ 0.62 ^a
20	2.60 $\pm$ 0.83 ^a	2.66 $\pm$ 0.87 ^a	1.61 $\pm$ 0.60 ^a
40	3.63 $\pm$ 0.54 ^a	4.02 $\pm$ 1.03 ^a	2.11 $\pm$ 0.51 ^a
60	4.04 $\pm$ 0.51 ^b	3.96 $\pm$ 0.32 ^a	2.62 $\pm$ 0.65 ^a
80	3.24 $\pm$ 0.59 ^a	3.6 $\pm$ 1.40 ^a	2.23 $\pm$ 0.52 ^a

Note: Numbers followed by the same letter in the same column are not significantly different at the $P < 0.05$ level of significance.

The final result of increased photosystem activity I in form of NADPH is equivalent to 3 ATP. NADPH can be used to provide reducing agents, usually hydrogen atoms, for biosynthetic and oxidation-reduction reactions involved in protection against the toxicity of reactive oxygen species (ROS), thus enabling glutathione (GSH) regeneration (Jomova et al., 2024). As a result, the leaves will also be greener because chlorophyll a has bluish green color (Zhang et al., 2021). The higher the colchicine concentration up to 60 μM , the more photosynthates are produced. Cells that are formed need a lot of hexoses for growth and development. With increased photosynthates due to the rise of chlorophyll a, increased hexoses are used to form new shoots as can be seen in **Table 1**. The number of shoots tends to increase with increasing concentration of colchicine up to 60 μM . It is assumed that hexoses resulting from photosynthetic activity will increase. Leaves that exposed to the sun possess sun-type chloroplasts that are adapted to high rates of photosynthetic quantum conversion (Lichtenthaler, 2021).

Polyploid induction is reported to increase chlorophyll content in some plants including *Pogostemon cablin* (Wu & Li, 2013), *Stevia reboudiana* (Zhang et al., 2018), *Artemisia annua* (Yunus et al., 2018), *Melissa officinalis* (Bharati et al., 2023), and *Begonia* (Xie et al., 2024). However, it was reported that colchicine-induced polyploid in *Gladiolus grandiflorus* decreased in the chlorophyll content (Manzoor et al., 2018), supporting the result of this study. Reducing total chlorophyll content in induced tetraploid plants occurred due to structural modifications, such as disintegration in the lamellar or thylakoid membrane of chloroplast, which affect the synthesis of chlorophyll (Xu et al., 2010).

Plant morphology can be used to analyze the ploidy level of a plant species in regard of the size, shape, color of plant parts in comparison to those of the diploid plants. The observation on both plant morphology and physiology is important to determine the response of plants to the treatment expressed on the phenotypes (Rao et al., 2019). The effects of 0.05% colchicine extract applied on *V. sumatrana* Schltr. for 24 hours, was studied for some morphological parameters. The results showed that colchicine had significant effects on cell length, cell width, nucleus diameter and leaf number. However, it had no significant effect on plantlet height, stem diameter and shoot number (Aini et al., 2015). This corresponds to the present study, in which colchicine has no significant effect on leaf width, leaf length and shoot number of *V. bensonii*.

The increase in chromosome number applied with colchicine is not able to significantly increase leaf width, leaf length and shoot number of both *V. bensonii* and *V. sumatrana*. Although this is probably due to the short period of observation time, it is also assumed that the increase in the chromosome number

makes the interphase phase takes longer, which slows down the cell division process (Aini et al., 2015).

Similarly, Kasmiyati et al. (2020) found that colchicine application to induce polyploidy in *Artemisia cina* shows no significant effect on some morphological and physiological characters due to several factors. The effects of colchicine in inducing polyploidization in some other plant species were also reported, such as those in *Glycyrrhiza glabra* (Moghbel et al., 2015), *Gladiolus grandifloras* (Manzoor et al., 2018), *Neolamarckia cadamba* (Eng et al., 2021), and *Centella asiatica* (Surson et al., 2024). These described the effects of colchicine in polyploidization not only regarding the concentration, but also the method and duration of application, and observed some genetic parameters of the treated plants.

CONCLUSIONS

Colchicine has effects only on plant height and chlorophyll a content of *V. bensonii*. No effective concentration of colchicine for leaf width, leaf length, leaf number, shoot number, chlorophyll b and total chlorophyll contents is found. With respect to plant height, control treatment shows the most effective effect. Meanwhile, colchicine concentration of 60 μM is found most effective on the chlorophyll a content. In short, it can be implied that colchicine has potential application to create mutants of better performance of *V. bensonii*. Further study on the duration and method of colchicine application is necessarily carried out on the improvement of this orchid species.

ACKNOWLEDGEMENTS

The authors are very grateful to the Institution of Research and Public Service, Universitas Jenderal Soedirman, Purwokerto for funding this project through the scheme of Riset Terapan Unggulan with contract number of T/527/UN23.18/PT.01.03/2021. High appreciation is also addressed to Supriyono for laboratory work assistance.

REFERENCES

- Aini, H., Mansyurdin, & Suwirmen. (2015). PLB induction of wild *Vanda sumatrana* Schltr. on MS media supplemented with BAP and NAA and ploidyisation by colchicine treatment. *Jurnal Biologi Universitas Andalas*, 4, 208-215.
- Argueless, E.D.L.R. (2025). First report of microalgae associated with velamen aerial roots of epiphytic orchids in the Philippines. *Journal of Tropical Life Science*, 15(2), 223-234.
- Bharati, R., Gupta, A., Novy, P., Severova, L., Sredl, K., Ziarovska, J., & Fernandez-Cusimamani, E. (2023). Synthetic polyploid induction influences morphological, physiological, and photosynthetic characteristics in *Melissa officinalis* L. *Frontiers in Plant Science*, 14(1332428), 1-15.

- Chazaux, M., Schiphorst, C., Lazzari, G., & Caffarri, S. (2022). Precise estimation of chlorophyll a, b and carotenoid content by deconvolution of the absorption spectrum and new simultaneous equations for Chl determination. *The Plant Journal*, 109, 1630-1648.
- Chung, H., Shi, S., Huang, B., & Chen, J. (2017). Enhanced agronomic traits and medicinal constituents of autotetraploids in *Anoectochilus formosanus* Hayata, a top-grade medicinal orchid. *Molecules* 22, 1-13.
- Corneillie, S., Storme, N.D., Acker, R.V., Fangel, J.U., Bruyne, M.D., Rycke, R.D., Geelen, D., Willats, W.G.T., Vanholme, B., & Boerjan, W. (2019). Polyploidy affects plant growth and alters cell wall composition. *Plant Physiology*. 179, 74-87.
- Dhyani, P., Quispe, C., Sharma, E., Bahukhandi, A., Sati, P., Attri, D.C., Szopa, A., Sharifi-Rad, J., Docea, A.O., Mardare, I., Calina, D., & Cho, W.C. (2022). Anticancer potential of alkaloids: a key emphasis to colchicine, vinblastine, vincristine, vindesine, vinorelbine and vincamine. *Cancer Cell International: Home page*, 22(206), 1-20.
- Dwiati, M., & Susanto, A.H. (2025). Adventitious shoot growth in *Grammatophyllum scriptum* var. Leopard Blume under colchicine and oryzalin applications. *BIO Web of Conference*, 158, 03014.
- Eng, W.H., Ho, W.S., & Ling, K.H. (2021). In vitro induction and identification of polyploid *Neolamarckia cadamba* plants by colchicine treatment. *Peer J*, DOI 10.7717/peerj.12399, 1-26.
- Guo, G., & Jeong, B.R. (2021). Explant, medium, and plant growth regulator (PGR) affect induction and proliferation of callus in *Abies koreana*. *Forest*, 12(1388), 1-14.
- Huy, N.P., Luan, V.Q., Tung, H.T., Hien, V.T., Ngan, H.T.M., Duy, P.N., & Nhut, D.T. (2019). In vitro polyploid induction of *Paphiopedilum villosum* using colchicine. *Sci. Hort.*, 252, 283-290.
- Jomova, K., Alomar, S.Y., Alwasel, S.H., Nepovimova, E., Kuca, K., & Valko, M. (2024). Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Archives of Toxicology*, 98, 1323-1367.
- Kasmiyati, S., Kristiani, E.B.E., & Herawati, M.M. (2020). Effect of induced polyploidy on plant growth, chlorophyll and flavonoid content of *Artemisia cina*. *Biosaintifika: Journal of Biology & Biology Education*, 12(1), 90-96.
- Kirankumar, H., Nataraj, S.K., Rajasekharan, P.E., Souravi, K. & Hanumanthappa, M. (2021). An insite into the *Vanda* genera: *Vanda's* natures wonder. *Advances in Crop Science and Technology*, 9(10), 1-8.
- Kumar, K. (2024). In vitro induction of polyploidy in ginger (*Zingiber officinale* Rosc.). Doctoral Thesis. Department of Biology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, India.
- Li, C., Zhang, M., Qi, N., Liu, H., Zhao, Z., Huang, P., & Liao, W. (2022). Absciscic acid induces adventitious rooting in cucumber (*Cucumis sativus* L.) by enhancing sugar synthesis. *Plants*, 11(2354), 1-11.
- Lichtenthaler, H.K., (2021). Multi-colour fluorescence imaging of photosynthetic activity and plant stress. *Photosynthetica*, 59, 4-20.
- Manzoor, A., Ahmad, T., Bashir, M.A., Baiq, M.M.Q., Quresh, A.A., Shah, M.K.N., & Hafiz, I.A. (2018). Induction and identification of colchicine induced polyploidy in *Gladiolus grandiflorus* „White Prosperity“. *Folia Horticulturae*, 30(2), 307-319.
- Moghbel, N., Borujeni, M.K. & Bernard, F. (2015). Colchicine effect on the DNA content and stomata size of *Glycyrrhiza glabra* var. Glandulifera and *Carthamus tinctorius* L. cultured in vitro. *Journal of Genetic Engineering and Biotechnology*, 13(1), 1-6.
- Mohammadi, V., Talebi, S., Ahmadnasab, M., & Mollahassanzadeh, H. (2023). The effect of induced polyploidy on phytochemistry, cellular organelles and the expression of genes involved in thymol and carvacrol biosynthetic pathway in thyme (*Thymus vulgaris*). *Frontiers in Plant Science*, 14(1228844), 1-10.
- Rao, S., Kang, X., Li, J., & Chen, J. (2019). Induction, identification and characterization of tetraploidy in *Lycium ruthenicum*. *Breeding Science*, 69(1), 160-168.
- Sukanto, D.S., Danuji, S., Putri, H.R., & Komaria, N. (2025). Colchicine-induced phenotypic alterations in *Dendrobium* 'Transient White Rika' and 'Florenza': valuable material for genetics-based learning modules. *Jurnal Penelitian Pendidikan IPA*, 11(4), 542-549.
- Surson, S., Shitthaphanit, S., & Wongjerson, K. (2024). Effective colchicine-induced polyploid induction in *Centella asiatica* (L.) Urban. *plant cell and tissue culture techniques*, 159(33), 1-11.
- Wu, Y. & Li, M. (2013). Induction of tetraploid plants of *Pogostemon cablin* (Blanco) and its quality evaluation. *Pharmacognosy Journal*, 5, 281-285.
- Xie, N., Zhao, Y., Huang, M., Chen, C., Cao, C., Wang, J., Shi, Z., & Gao, J. (2024). Polyploid induction and identification of *Begonia* × *benariensis*. *Horticulturae*, 10(47), 1-13.
- Xu, L., Najeeb, U., Naeem, M.S., Daud, M.K., Cao, J.S., Gong, H.J., Shen, W.Q. & Zhou, W.J.

- (2010). Induction of tetraploidy in *Juncus effusus* by colchicine. *Biologia Plantarum*, 54(4), 659-663.
- Yunus, A., Parjanto, Samanhudi, Hikam, M.P., & Widyastuti, Y. (2018). Polyploid response of *Artemisia annua* L. to colchicine treatment. *IOP Conf. Series: Earth and Environ. Sci.*, 142.
- Zhang, H., An, S., Hu, J., Lin, Z., Liu, X., Bao, H., & Chen, R. (2018). Induction, identification and characterization of polyploidy in *Stevia rebaudiana* Bertoni. *Plant Biotechnology*, 35, 81–86
- Zhang, J., Sui, C., Liu, H., Chen, J., Han, Z., Yan, Q., Liu, S., & Liu, H. (2021). Effect of chlorophyll biosynthesis-related genes on the leaf color in hosta (*Hosta plantaginea* Aschers) and tobacco (*Nicotiana tabacum* L.). *BMC Plant Biology*, 21(45), 1-14.