INTRODUCTION

Stevia (*Stevia rebaudiana* Bert.) is a natural sweetener plant used as a sugar substitute in commercial and healthcare industries that is widely used for illness therapy because it has anti-cancer, anti-hypersensitive, anti-hyperglycemic, and anti-microbial activities. Steviol glycosides content in stevioside, rebaudioside A, and rebaudioside C forms of stevia has a sweetness 200 to 300 times higher than cane sugar. Stevia also contains total flavonoids with high antioxidant activity (Amarakoon, 2021; Moongngarm et al., 2022; Peteliuk et al., 2021).

Stevia grows optimally in semi-humid areas with an average temperature of 6°C and rainfall of 1500-1800 mm/year. In a suitable environment, the flowering age of stevia occurs about 50 to 100 days after planting. Stevia is harvested at the flowering phase when its steviol glycol content reaches peak levels. Stevia could be propagated using seeds, vegetatively, and tissue culture, but propagation by seeds has some major constraints, namely low production, viability, and germination of stevia seeds. Vegetative propagation with stem cuttings is also not easy because the emergence and growth of shoots are influenced by several factors, such as the part of the plant used, the length of the cuttings, the number of internodes of the cuttings and the time of making the cuttings (season). The most widely practiced stevia propagation is through tissue cultures (Gunasena et al., 2021). The increasing demand for stevia should be balanced with improvements in planting materials and cultivation techniques so that stevia yields an increase (Amarakoon, 2021). awangmangu local variety of stevia has stevioside levels of 11.38-16.09% and Rebaudioside A 1.37-1.85%. The stevioside content of local varieties is within the range of stevioside stevia from Paraguay, which is 5.1-21%, while the rebaudioside A content of local varieties is lower than the range of rebaudioside A stevia from Paraguay (0-12%) (Penner et al., 2004). Efforts are needed to improve the quality of stevia.

Planting material improvements could be done using the superior seeds obtained from the results of crosses, induction of mutations, and assembly of the new high-yielding varieties due to high biomass yields and levels of secondary metabolite compounds. Crossbreeding of stevia is difficult due to stevia's self-incompatibility, leading to a low probability of successful self-pollination. In addition, obtaining viable seeds is challenging due to low germination capacity (Sinta et al., 2018). Plant breeding with polyploidization, irradiation, or genome editing is one of many solutions to increase plant diversity and seed availability in plants that are difficult to develop conventionally. Several plant mutation induction (polyploidization) could produce polyploid plants with higher growth and secondary metabolite compound content (Adabiyah et al., 2019; Gantait & Mukherjee, 2021).

Polyploid plants experience chromosome number changes from their origin due to mutation induction. There are two kinds of mutation induction agents (mutagens), namely physical mutagens (gamma rays, x-rays, beta rays, and UV rays) and chemical mutagens (EMS, DES, colchicine, and oryzalin) (Ermayanti et al., 2018; Lestari, 2021). Research related to mutation induction effect on the content of plant secondary metabolite compounds was carried out at *Dendrobium officinale* (Pham et al., 2019), *Solanum bulbocastanum* (Caruso et al., 2013), *Physalis peruviana* L. (Çömlekçioğlu & Özden, 2020), *Melissa officinalis* (Talei & Fotokian, 2020), *Capsicum frutescens* (Pliankong et al., 2017), *Artemisia cina* (Kasmiyati et al., 2020) and *Cnidium officinale* (Kim et al., 2021). Oryzalin is a safer mutation agent because it does not inhibit the vegetative growth of plants (Surya et al., 2016). Mutation induction using oryzalin in local Tawangmangu stevia plants is expected to increase the diversity of stevia plants with properties different from those of diploid stevia plants.

effective methods for estimating tetraploid plants before transplantation to the field.

Stevia plants that were oryzalin-induced showed differences in morphological characteristics from control plants. One of the morphological characteristics seen is the difference in the size of the induced stevia stomata with the control, and some treatments show differences in stomatal density. It is in line with the results of research (Rahmi et al., 2019; Ridwan et al., 2018; Ridwan & Witjaksono, 2020; Wang et al., 2020). As the microtubules destabilizer (microtubules inhibitor), Oryzalin disrupts and inhibits the function of microtubules, consequently inhibiting plant growth. Microtubules are fundamental components that support the diverse morphology and dynamics of various cell types, cell cycles, and developmental stages of plants and animals. Most microtubule inhibitors produce cytotoxicity in plant and animal cells. Oryzalin causes aberrant growth with milder effects than higher microtubule depolymerizing agents and also induces anisotropic expansion of epidermal cells (Ishida et al., 2021; Roll-Mecak, 2020). Stomata are one of the plant characters affected by chromosome manipulation and the polyploidization process, where changes in stomatal size and density due to polyploidization can affect the photosynthetic process of plants (Handayani et al., 2023).

Erboğa et al. (2021) stated that oryzalin usage increased the length and width of the leaves stomatal and decreased the stomatal density. The chromosome number increased due to polyploidization increases in the stomatal size, which caused decreases in the stomatal density. Tetraploid plants have an increase in the number of chloroplasts in stomatal guard cells twice from diploid. Thus, stomatal density and size can serve as an index to distinguish tetraploid L. ruthenicum from its diploid because the size and density of stomata, as well as the frequency of chloroplast content of polyploidized plants, are inversely proportional to their ploidy level (Kara & Doğan, 2022; Rao et al., 2019). In this study, the treatment of oryzalin concentration and immersion time caused increases in the stomatal size but did not affect the stomatal density of stevia leaves. There was no increase in the size of stomatal guard cells and the size of non-stomatal epidermal cells (Figure 4). It agrees with Šmarda et al. (2023) that the stomatal density of the polyploidization effect is not always lower, although most are lower than the diploid.

Table 2. Effect of concentration and duration of oryzalin immersion on stomata of some morphologically polyploid stevia plants

Treatment	Stomata length	Stomatal diameter	Notes
Control	48.55	38.85	Normal
1.5µM-4hours (5)	51.99	42.73	no epidermal thickening occurs
1.5µM-6hours (13)	48.55	38.85	epidermal thickening occurs, cells are damaged
1.5µM-8hours (14)	61.10	38.91	epidermal thickening occurs
2.5µM-4hours (3)	55.98	42.39	epidermal thickening occurs, cells are damaged
2.5µM-6hours (3)	64.43	50.57	epidermal thickening occurs
2.5µM-8hours (15)	52.18	42.04	epidermal thickening occurs
3.5µM-4hours (12)	61.57	49.10	epidermal thickening occurs
3.5µM-6hours (15)	55.67	40.78	no epidermal thickening occurs
3.5µM-8hours (5)	50.09	40.80	epidermal thickening occurs

Leaf colors

The various concentrations of oryzalin with three different immersion times influenced the color of stevia leaves (Table 3). The control stevia had a dark sea green leaf color, while the treated stevia had 14 different leaf colors from the control leaf. The three most common stevia leaf colors were dark sea green (29 individuals), asparagus green (19 individuals), and smoke green (16 individuals). It is presumed that the color difference in stevia treatment results was affected by pigments or compounds contained in stevia leaves. One of the organs that affected the green color of the leaves was the stomata, which contain green pigments (chlorophyll).

Zeng et al. (2019) stated that the doubling chromosomes due to polyploidization causes modification of vegetative characteristics such as a decrease in plant height, thicker leaves with a darker green color, and an increase in root diameter accompanied by root number and length decrease. The dark green color of leaves treated with mutation agents can be used as an indicator that the polyploidization process occurs in plants due to the presence of higher chlorophyll content (Bharati et al., 2023; Handayani et al., 2023; Yadav et al., 2013). The results of this study indicate that some plants that have been treated with oryzalin concentration and immersion duration have darker leaf color compared to stevia leaves without treatment (control).

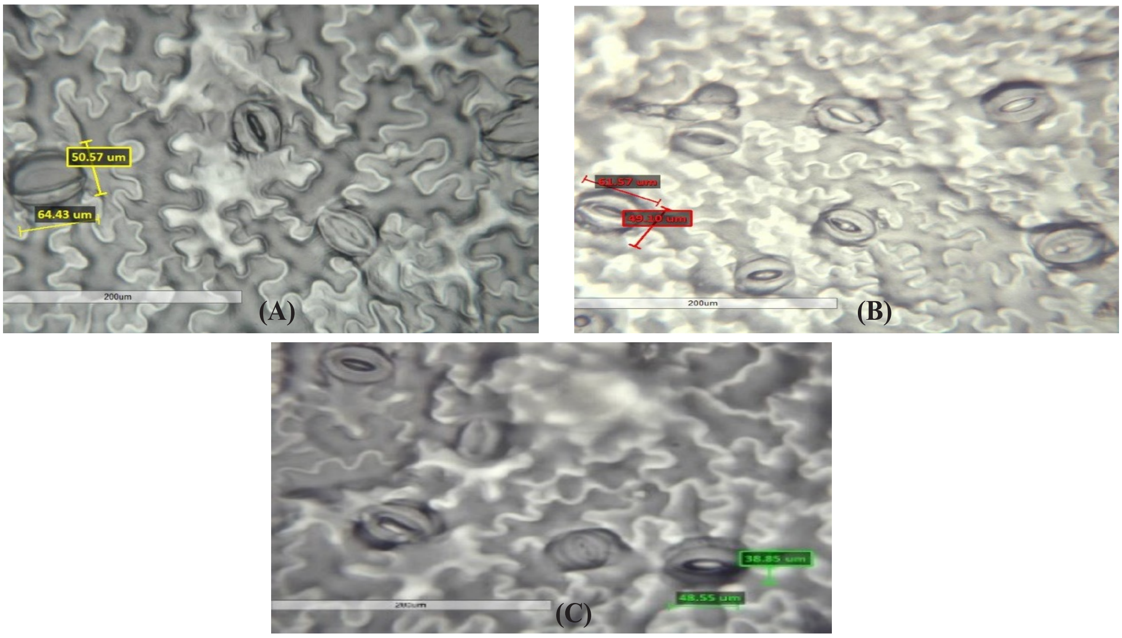

Figure 4. Effect of concentration and duration of oryzalin immersion on stevia stomata indicated as polyploid based on morphology. (A) 2.5µM-6hours (3); (B) 3.5µM-6hours (12); (C) Control or stomata of stevia leaves with out treatment. Total magnification 400x. Bar scale = 200 µm(micrometer). µM = micromolar.

Table 3. Color variation of stevia after treatment of oryzalin concentration and immersion time

Colors	Control	Treatment								
		1.5µM -4h	1.5µM -6h	1.5µM -8h	2.5µM -4h	2.5µM -6h	2.5µM -8h	3.5µM -4h	3.5µM -6h	3.5µM -8h
Dark sea green	15		3		4	5	5	5	4	3
Asparagus green		2	4	1	1	4	2	2	2	1
Medium sea green			3	1				1		
Green smoke		6	1	2	2		1	1	1	2
Light green		3	1		1	1			1	1
Sea green					2	1				
Dark olive green		1					3	2		
Bitter green		1								
Cactus green			1	1	2	1			2	
Verdun green							1		1	
Pale green			1			1				1
Olive drab					1	1				
Sirroco green				1						
Yellow-green								1		
Yellowish brown								1		

Notes: µM = microMolar; h = hours

Ploidy level

Based on the treated stevia plant morphology that showed different growth indications from the control plants, stevia in the 3.5µM-6h treatment with sample number 7 was further tested by flow cytometry. The results of flow cytometry showed that the tested stevia plants did not show polyploidy. The results of the flow cytometry test of diploid plants (control) and treated plants display a peak level at a height of 100 (Figure 5). Morphological changes in plants indicate polyploids can occur due to genetic changes due to the induction of mutation agents or the influence of physiological, biochemical, and transcriptomic changes (Ruiz et al., 2020).

Mutation induction is an attempt to change the morphology and traits of plants due to genetic changes. Successful induction requires a synergistic pairing of the penetration efficiency of microtubulin inhibitors (antimitotic agents) and plant meristematic tissues. In addition, success depends on the length of exposure, the concentration of antimitotic agent used, and in in-vitro applications depends on the interaction between basal media and growth regulators (Mullins et al., 2021; Touchell et al., 2020). The success of mutation induction is influenced by determining the duration and dose of exposure to mutagen compounds that inhibit the formation of microtubule compounds. The optimal immersion time in oryzalin solution differs with different concentrations but is around one day for *Lilium rosthornii* with a concentration of 0.01% (34.6 µM) (Wang et al., 2020). In addition, de Carvalho et al. (2016) also stated that handling techniques and the condition of the plants at the time of treatment application also affect the sensitivity of the plants to the given oryzalin because it becomes a bridge to facilitate the penetration of the antimitotic agent into the plant tissue.

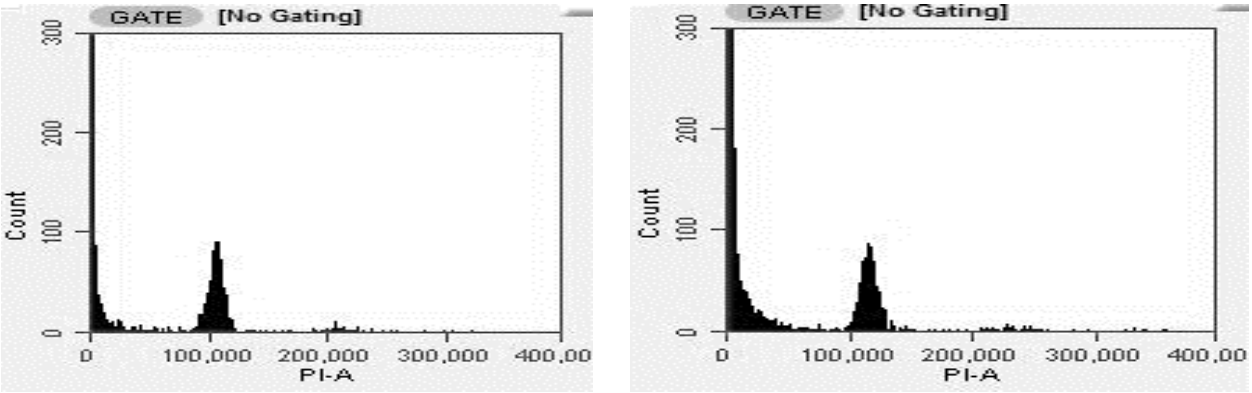

Figure 5. Results of flow cytometric analysis of diploid stevia plants (control) (a) and selected treated stevia plants (2.5µM-6hours sample number 3) (b)

CONCLUSION

Oryzalin concentration up to 3.5µM in 6 hours of immersion time was safe to induce mutation because it produced 67% live stevia. The oryzalin combination treatment applied to the stevia variety Tawangmangu generated morphological growth that indicates polyploidized including height, number of internodes, internode length, stem diameter, leaf size, leaf thickness, leaf color, stomata, and stem diameter. In addition, there were variations in growth, such as chimeras, rosette growth, and leaf splitting. However, further testing using flow cytometry showed that the treatment of concentration and duration of oryzalin immersion directly on vegetative material did not produce polyploid stevia individuals. Further research on the higher oryzalin concentration, longer immersion time, and stevia meristematic parts treated to obtain tetraploid stevia.

AUTHOR CONTRIBUTION

D.S. designed, conducted, collected research data, analyzed, interpreted data, drafted, and finalized the manuscript; P. and S.A. provided guidance, advice, input and supervision during the research process and manuscript drafting.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Center for Research on Medicinal Raw Materials and Traditional Medicines - National Research and Innovation Agency (PR BBOOT - BRIN) and Health Research and Development Agency - Ministry of Health for permission and support. This research is also supported by research facilities, as well as scientific and technical support from the Genomic Characterization Laboratory at BRIN". The authors also extend their gratitude to Dyah Subositi, Harto Widodo, and all PR BBOOT-BRIN research colleagues who provided the authors with ideas, support, advice, and insight.

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

REFERENCES

Adabiyah, R., Ratnadewi, D., & Ermayanti, T. M. (2019). Evaluasi Pertumbuhan *Stevia rebaudiana* Bert. Tetraploid Secara In Vitro dan di Lapang untuk Produksi Steviosida dan Rebaudiosida-A. *Jurnal Biologi Indonesia*, 15(2), 153–165.

Amarakoon, S. (2021). *Stevia rebaudiana*-A review on agricultural, chemical, and industrial applications. *Journal of Nature and Applied Research*, 1(1), 2792–1352. www.natark.com

Arunachalam, V., Salgaonkar, D. C., Kevat, N. V., Walawalkar, B. V., & Das, B. (2022). Quantification of betacyanin content variation of amaranth varieties by an Android App, colorimeter, and infrared spectroscopy. *Chinese Journal of Analytical Chemistry*, 50(10), 100145. https://doi.org/10.1016/j.cjac.2022.100145

Bae, S. J., Islam, M. M., Kim, H. Y., & Lim, K. B. (2020). Induction of Tetraploidy in Watermelon with Oryzalin Treatments. *Horticultural Science and Technology*, 38(3), 385–393. https://doi.org/https://doi.org/10.7235/HORT.20200037

Bharati, R., Fernández-Cusimamani, E., Gupta, A., Novy, P., Moses, O., Severová, L., Svoboda, R., & Šrédl, K. (2023). Oryzalin induces polyploids with superior morphology and increased levels of essential oil production in *Mentha spicata* L. *Industrial Crops and Products*, 198(2019), 116683. https://doi.org/10.1016/j.indcrop.2023.116683

Caruso, I., Piaz, F. D., Malafronte, N., De Tommasi, N., Aversano, R., Zottele, C. W., Scarano, M. T., & Carputo, D. (2013). Impact of ploidy change on secondary metabolites and photochemical efficiency in *Solanum bulbocastanum*. *Natural Product Communications*, 8(10), 1387–1392. https://doi.org/10.1177/1934578x1300801011

Çömlekçioğlu, N., & Özden, M. (2020). Effects of colchicine application and ploidy level on fruit secondary metabolite profiles of goldenberry (*Physalis peruviana* L.). *Applied Ecology and Environmental Research*, 18(1), 289–302. https://doi.org/10.15666/aeer/1801_289302

de Carvalho, M. de J. d. S., Gomes, V. B., Souza, A. da S., Aud, F. F., Santos-Serejo, J. A., & Oliveira, E. J. (2016). Inducing autotetraploids in cassava using oryzalin and colchicine and their in vitro morphophysiological effects. *Genetics and Molecular Research*, 15(2), 1–14. https://doi.org/10.4238/gmr.15028281

Deans, L. E., Palmer, I. E., Touchell, D. H., & Ranney, T. G. (2021). In Vitro Induction and Characterization of Polyploid *Hydrangea macrophylla* and *H. serrata*. *HortScience*, 56(6), 709–715. https://doi.org/10.21273/HORTSCI15783-21

Erboğa, M., Doğan, O., & Kara, Z. (2021). The Effects of Nitrogen Protoxide and Orizalin on Promotion of Polyploidy in Grapes. *Selcuk Journal of Agricultural and Food Sciences*, 35(3), 244–248. https://doi.org/10.15316/SJAFS.2021.253

Ermayanti, T. M., Wijayanta, A. N., & Ratnadewi, D. (2018). Induksi Poliploidi pada tanaman talas (*Colocasia esculenta* (L .) Schott) Kultivar Kaliurang dengan perlakuan kolkisin secara in vitro. *Biologi Indonesia*, 14(1), 91–102.

Friska, M., & Daryono, B. S. (2017). Karakter Fenotip Jahe Merah (*Zingiber officinale* Roxb. var rubrum Rosc.) Hasil Poliploidisasi dengan Kolkisin. *Al-Kauniyah: Jurnal Biologi*, 10(2), 91–97. https://doi.org/10.15408/kauniyah.v10i2.4813

Gantait, S., & Mukherjee, E. (2021). Induced autopolyploidy—a promising approach for enhanced biosynthesis of plant secondary metabolites: an insight.

In *Journal of Genetic Engineering and Biotechnology* (Vol. 19, Issue 4, pp. 1–13). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1186/s43141-020-00109-8

Gunasena, M. D. K. M., Sachinthanie Senarath, R. M. U., & Senarath, W. T. P. S. K. (2021). A Review on Chemical Composition, Biosynthesis of Steviol Glycosides, Application, Cultivation, and Phytochemical Screening of *Stevia rebaudiana* (Bert.) Bertoni. *Journal of Pharmaceutical Research International*, 33, 85–104. https://doi.org/10.9734/jpri/2021/v33i29B31593

Handayani, T., Prawestri, A. D., Rahayu, R. S., & Leksonowati, A. (2023). Oryzalin-Induced Taro (*Colocasia esculenta* L.) Tetraploid and Diploid Assessment for Growth and Agronomic Traits. *SABRAO Journal of Breeding and Genetics,* 55(1), 163–174. https://doi.org/10.54910/sabrao2023.55.1.16

Handayani, T., Witjaksono, & Nugraheni, K. U. (2017). Induksi Tetraploid Pada Tanaman Jambu Biji Merah (*Psidium guajava* L.) secara In Vitro. *Jurnal Biologi Indonesia*, 13(2), 271–278. https://doi.org/10.47349/jbi/13022017/271

Ishida, T., Yoshimura, H., Takekawa, M., Higaki, T., Ideue, T., Hatano, M., Igarashi, M., Tani, T., Sawa, S., & Ishikawa, H. (2021). Discovery, characterization, and functional improvement of kumamonamide as a novel plant growth inhibitor that disturbs plant microtubules. *Scientific Reports*, 11(1), 6077. https://doi.org/10.1038/s41598-021-85501-1

Kara, Z., & Doğan, O. (2022). Reactions of Some Grapevine Rootstock Cuttings to Mutagenic Applications. *Selcuk Journal of Agricultural and Food Sciences,* 36(2), 238–246. https://doi.org/10.15316/SJAFS.2022.031

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. https://doi.org/10.15294/biosaintifika.v12i1.22548

Kim, H. E., Han, J. E., Lee, H., Kim, J. H., Kim, H. H., Lee, K. Y., Shin, J. H., Kim, H. K., & Park, S. Y. (2021). Tetraploidization increases the contents of functional metabolites in *Cnidium officinale. Agronomy*, 11(1561), 1–15. https://doi.org/10.3390/agronomy11081561

Langhans, M., Niemes, S., Pimpl, P., & Robinson, D. G. (2009). Oryzalin bodies: In addition to its anti-microtubule properties, the dinitroaniline herbicide oryzalin causes nodulation of the endoplasmic reticulum. *Protoplasma*, 236(1–4), 73–84. https://doi.org/10.1007/s00709-009-0059-2

Lestari, E. G. (2021). Aplikasi Induksi Mutasi Untuk Pemuliaan Tanaman Hias. *Ilmu-Ilmu Hayati*, 21(3), 335–344.

Moongngarm, A., Sriharboot, N., Loypimai, P., & Moontree, T. (2022). Ohmic heating-assisted water extraction of steviol glycosides and phytochemicals from *Stevia rebaudiana* leaves. *LWT - Food Science and Technology*, 154, 112798. https://doi.org/10.1016/j.lwt.2021.112798

Morejohn, L. C., Bureau, T. E., Molè-Bajer, J., Bajer, A. S., & Fosket, D. E. (1987). Oryzalin, a dinitroaniline herbicide, binds to plant tubulin and inhibits microtubule polymerization in vitro. *Planta*, 172(2), 252–264. https://doi.org/10.1007/BF00394595

Mullins, E., Bresson, J., Dalmay, T., Dewhurst, I. C., Epstein, M. M., Firbank, L. G., Guerche, P., Hejatko, J., Moreno, F. J., Naegeli, H., Nogué, F., Sánchez Serrano,

J. J., Savoini, G., Veromann, E., Veronesi, F., Casacuberta, J., Lenzi, P., Munoz Guajardo, I., Raffaello, T., & Rostoks, N. (2021). In vivo and in vitro random mutagenesis techniques in plants. *EFSA Journal*, 19(11), e06611. https://doi.org/10.2903/j.efsa.2021.6611

Penner, R., Shanks, T., Timcke, K., Krigbaum, J., & Uno, J. (2004). Stevia from Paraguay (Vol. 1).

Peteliuk, V., Rybchuk, L., Bayliak, M., Storey, K. B., & Lushchak, O. (2021). Natural sweetener stevia rebaudiana: Functionalities, health benefits and potential risks. *EXCLI Journal*, 20, 1412–1430. https://doi.org/http://dx.doi.org/10.17179/excli2021-4211

Pham, P. L., Li, Y. X., Guo, H. R., Zeng, R. Z., Xie, L., Zhang, Z. S., Chen, J., Su, Q. L., & Xia, Q. (2019). Changes in morphological characteristics, regeneration ability, and polysaccharide content in tetraploid *Dendrobium officinale*. *HortScience*, 54(11), 1879–1886. https://doi.org/10.21273/HORTSCI14310-19

Pliankong, P., Ard, P. S., & Wannakrairoj, S. (2017). Effects of Colchicine and Oryzalin on Polyploidy Induction and Production of Capsaicin in *Capsicum frutescens* L . *Thai Journal of Agricultural Science*, 50(2), 108–120.

Rahman, W., Al Hafiizh, E., Muji Ermayanti, T., Ellfy Rantau, D., & A. Lelono, A. (2017). Acclimation and Agronomic Performance of Polyploids Clones of *Artemisia annua* L. *Jurnal Biologi Indonesia*, 13(1), 34–42. https://doi.org/10.47349/jbi/13012017/34

Rahmi, P., Witjaksono, & Ratnadewi, D. (2019). Induksi Poliploidi Tanaman Kangkung (*Ipomoea aquatica* Forssk.) Kultivar Salina In Vitro dengan Oryzalin. *Jurnal Biologi Indonesia*, 15(1), 1–8. https://doi.org/10.47349/jbi/15012019/1

Rao, S., Kang, X., Li, J., & Chen, J. (2019). Induction, identification, and characterization of tetraploidy in *Lycium ruthenicum*. *Breeding Science*, 69(1), 160–168. https://doi.org/10.1270/jsbbs.18144

Ridwan, R., Handayani, T., Riastiwi, I., & Witjaksono, W. (2018). Tetraploid teak seedling was more tolerant to drought stress than its diploid seedling. *Jurnal Penelitian Kehutanan Wallacea*, 7(1), 1. https://doi.org/10.18330/jwallacea.2018.vol7iss1pp1-11

Ridwan, R., & Witjaksono, W. (2020). Induction of autotetraploid Moringa plant (*Moringa oleifera*) using oryzalin. *Biodiversitas Journal of Biological Diversity*, 21(9), 4086–4093. https://doi.org/10.13057/biodiv/d210920

Rohmah, L. B. (2019). Karakter Stomata dan Fenotipik Tanaman Stevia (*Stevia rebaudiana* Bertoni.) Hasil Induksi Oryzalin secara In-Vitro.

Roll-Mecak, A. (2020). The Tubulin Code in Microtubule Dynamics and Information Encoding. *Developmental Cell*, 54(1), 7–20. https://doi.org/10.1016/j.devcel.2020.06.008

Ruiz, M., Oustric, J., Santini, J., & Morillon, R. (2020). Synthetic Polyploidy in Grafted Crops. *Frontiers in Plant Science*, 11(November), 1–19. https://doi.org/10.3389/fpls.2020.540894

Silalahi, C. B., Sinuraya, M., Hanafiah, D. S., & Sipayung, R. (2020). The influence of Oryzalin concentrations on the plant growth of two tomato (*Solanum lycopersicum* L.) varieties. *IOP Conference Series: Earth and Environmental Science*, 454(1), 012161. https://doi.org/10.1088/1755-1315/454/1/012161

Sinta, M. M., Wiendi, N. M. A., & Aisyah, S. I. (2018). Induksi mutasi Stevia rebaudiana dengan perendaman kolkisin secara in vitro. *E-Journal Menara Perkebunan*, 86(1), 1–10. https://doi.org/10.22302/iribb.jur.mp.v1i1.277

Šmarda, P., Klem, K., Knápek, O., Veselá, B., Veselá, K., Holub, P., Kuchař, V., Šilerová, A., Horová, L., & Bureš, P. (2023). Growth, physiology, and stomatal parameters of plant polyploids grown under ice age, present-day, and future CO_2 concentrations. *New Phytologist,* 239(1), 399–414. https://doi.org/10.1111/nph.18955

Surya, M. I., Ismaini, L., Destri, D., & Normasiwi, S. (2016). An Effort of Mutation Breeding by Oryzalin and Gamma Rays on Wild Raspberry (*Rubus* sp.) in Cibodas Botanical Garden. *Biosaintifika: Journal of Biology & Biology Education*, 8(3), 331. https://doi.org/10.15294/biosaintifika.v8i3.6559

Talei, D., & Fotokian, M. H. (2020). Improving growth indices and productivity of phytochemical compounds in lemon balm (*Melissa officinalis* L.) through induced polyploidy. *Biotechnologia*, 101(3), 215–226. https://doi.org/10.5114/bta.2020.97880

Tang, Z. Q., Chen, D. L., Song, Z. J., He, Y. C., & Cai, D. T. (2010). In vitro induction and identification of tetraploid plants of Paulownia tomentosa. *Plant Cell, Tissue and Organ Culture,* 102(2), 213–220. https://doi.org/10.1007/S11240-010-9724-6/METRICS

Touchell, D. H., Palmer, I. E., & Ranney, T. G. (2020). In vitro Ploidy Manipulation for Crop Improvement. *Frontiers in Plant Science*, 11(June), 1–11. https://doi.org/10.3389/fpls.2020.00722

Wang, L.-J., Zhang, Q., Cao, Q.-Z., Gao, X., & Jia, G.-X. (2020). An efficient method for inducing multiple genotypes of tetraploids *Lilium rosthornii* Diels. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 141(3), 499–510. https://doi.org/10.1007/s11240-020-01807-4

Wen, Y., Liu, H., Meng, H., Qiao, L., Zhang, G., & Cheng, Z. (2022). In vitro Induction and Phenotypic Variations of Autotetraploid Garlic (*Allium sativum* L.) with Dwarfism. *Frontiers in Plant Science*, 13(June), 1–16. https://doi.org/10.3389/fpls.2022.917910

Yadav, A. K., Singh, S., Yadav, S. C., & Dhyani, D. (2013). Induction and morpho-chemical characterization of Stevia rebaudiana colchiploids. *Article in Indian Journal of Agricultural Sciences*, 83(2), 159–165. https://www.researchgate.net/publication/257098274

Zahumenická, P., Fernández, E., Šedivá, J., Žiarovská, J., Ros-Santaella, J. L., Martínez-Fernández, D., Russo, D., & Milella, L. (2018). Morphological, physiological and genomic comparisons between diploids and induced tetraploids in *Anemone sylvestris* L. *Plant Cell, Tissue and Organ Culture (PCTOC),* 132(2), 317–327. https://doi.org/10.1007/s11240-017-1331-3

Zeng, Q., Liu, Z., Du, K., & Kang, X. (2019). Oryzalin-induced chromosome doubling in triploid Populus and its effect on plant morphology and anatomy. *Plant Cell, Tissue and Organ Culture (PCTOC),* 138(3), 571–581. https://doi.org/10.1007/s11240-019-01654-y

Zhang, Y.-S., Chen, J.-J., Cao, Y.-M., Duan, J.-X., & Cai, X.-D. (2020). Induction of tetraploids in 'Red Flash' caladium using colchicine and oryzalin: Morphological, cytological, photosynthetic and chilling tolerance analysis. *Scientia Horticulturae*, 272(April), 109524. https://doi.org/10.1016/j.scienta.2020.109524

Zhou, H. wen, Zeng, W. dan, & Yan, H. bing. (2017). In vitro induction of tetraploids in cassava variety 'Xinxuan 048' using colchicine. *Plant Cell, Tissue and Organ Culture*, 128(3), 723–729. https://doi.org/10.1007/s11240-016-1141-z