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Evaluating the effect of plantlet age and Trichoderma spp. application on acclimatization of *Boehmeria nivea* L. Gaud

Abstract. One of the crucial stages in the ramie plant (*Boehmeria nivea* L. Gaud) tissue culture is the acclimatization phase, which transitions between the *in vitro* and *ex-vitro* environments. Determining the appropriate age of plantlets and providing nutrients in the substrate play significant roles in the success of acclimatization. This research is conducted to determine the most suitable age of plantlets for acclimatization and assess the impact of providing nutrients through *Trichoderma* spp. application on the growth of ramie seedlings during the acclimatization phase. The experiment was conducted between January and March 2023 at the Screenhouse Bale Tatanen, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, Sumedang. The experimental design used was a Randomized Complete Block Design (RCBD) with two factors: plantlet age with three levels (4, 8, and 12 weeks old) and *Trichoderma* spp. fertilizer application with two levels (without and with *Trichoderma*) with 4 replications. The results showed that the 4-week-old plantlets exhibited the best height, root number, root length, and leaf number growth. Additionally, the application of *Trichoderma* spp. increased the stem diameter of the ramie seedlings during the acclimatization phase.

Keywords: Acclimatization · *Boehmeria nivea* L. Gaud · Plantlet ages · *Trichoderma* spp.

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Introduction

Ramie is one of the annual plants that produce natural fibers. Its fibers have superior properties to other plant fibers, such as high water absorption, strong, long strands, good compatibility, and luster (Novarini & Sukardan, 2015; Muryanto et al., 2018). Ramie can be developed in Indonesia as an alternative fiber-producing crop to cotton. Indonesia's demand for cotton fiber is very high, at 700,000 tons or US$1 billion per year, mostly met by imports (Suparno, 2020).

The use of superior clones is essential in producing high-quality ramie. One of Indonesia's superior and high-yielding ramie is the Ramindo-1 clone, with a productivity of 2-2.7 tons/ha/year and adaptability to various altitudes (Suherman et al., 2017). Ramie cultivation in Indonesia often faces challenges because planting materials do not meet seedling standards (Purwati & Sudjindro, 2004).

Rhizomes used as planting materials require a long time for production and have a short storage life (Purwati, 2010; Mukherjee et al., 2018). Compared to conventional plant propagation methods like rhizomes, tissue culture propagation can produce many uniforms, virus-free seedlings rapidly (Alexopoulus & Petropoulus, 2021). Among the stages in tissue culture, the final step determining its success is the acclimatization phase (Hiti-Bandaralage et al., 2017). The success of in vitro seedling production greatly depends on the plantlets' ability to adapt and survive when transferred to the ex vitro environment (Saptari et al., 2017; Bag et al., 2019).

Prausová et al. (2015) found that the initial size of plantlets during the acclimatization process affects their survival rate, biomass increment, and leaf quantity. Older plantlets tend to be larger with more developed organs. However, a longer age does not necessarily make the plantlets more ready for acclimatization. Palee et al. (2012) discovered that 4 and 8-week-old plantlets had higher survival rates compared to 12-week-old plantlets in the case of *Stemona curtisii*. The shorter the age at which the plantlets can be acclimatized, the more efficient the in vitro seedling production process becomes as the production stages are shortened.

Fertilizer application during the acclimatization phase is crucial. The application of certain microbes, such as *Trichoderma* spp., can be a suitable option as it is known as a plant growth-promoting fungi (PGPF) and a biocontrol agent that enhances the survival rate and supports the growth of vulnerable plantlets (Sparta & Emilda, 2020). Fertilization significantly impacts plant growth during ex vitro acclimatization, allowing for a shorter period and making the plants ready to be transferred to a greenhouse or field faster (Pirata et al., 2022).

There is limited specific research discussing the influence of plantlet age and *Trichoderma* spp. application during the acclimatization stage, especially concerning ramie plants. No publications yet address the complete acclimatization process, including the optimum plantlet age for this stage. The present study aimed to evaluate the effect of plantlet age and *Trichoderma* spp. on the performance of ramie plantlets. It allows us to determine the appropriate plantlet age for acclimatization and evaluate the necessity of *Trichoderma* application during the acclimatization process.

Materials and Methods

Study sites and plant resources. The acclimatization experiment was conducted at the Screen House Bale Tatanen, Faculty of Agriculture, Universitas Padjadjaran, from February to March 2023. The preparation of planting materials, which included plantlets from tissue culture, was carried out at the Tissue Culture and Seed Technology Laboratory, Faculty of Agriculture, Universitas Padjadjaran.

Materials. The materials used were ramie plantlets of the Ramindo-1 clone obtained from tissue cultures 4, 8, and 12 weeks old. Soil medium was used with the ratio1:1:1 of soil, compost, and vermiculite, *Trichoderma* spp. fertilizer (GM *Trichoderma*), and distilled water. Equipment used in this experiment included culture bottles, spatulas, forceps, 10 cm diameter pots, plastic pots, buckets, calipers, meters, writing tools, planting tables, Thermo-hygrometers, chlorophyll meters (MC—100, Apogee Instruments, Inc.), measuring cups, cameras, and photo boxes.

Method. The experimental design used was an RCBD with a factorial arrangement of two factors: plantlet age (A) and *Trichoderma* spp. application (T). Plantlet age had three levels: 4 weeks (a1), 8 weeks (a2), and 12 weeks (a3); and *Trichoderma* spp. application had two levels:

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without *Trichoderma* application (t0) and with *Trichoderma* application (t1). There was a total of six treatment combinations, each replicated four times.

The plantlets used were obtained from the second subculture of axillary bud explants conducted at the Tissue Culture and Seed Technology Laboratory, Faculty of Agriculture, Universitas Padjadjaran. The *Trichoderma* fertilizer used was GMN *Trichoderma* fertilizer (PT. Trubus Prima) with registration number 03.03.2022.426. The media used were compost, soil, and vermiculite, which were sterilized by steam. The fertilizer application was conducted at a recommended dosage of 100 g per 25 kg of compost, which was then mixed with the modified media according to the study by Mukherjee et al. (2018), which was a 1:1:1 mixture of soil, compost, and vermiculite. In this study, 4 g of *Trichoderma* spp. fertilizer was mixed into 1 kg of compost. *Trichoderma* spp. was applied to the media one week before the acclimation experiment.

Plantlets with different age treatments were carefully removed from the bottles and rinsed with running water to remove any remaining media and prevent pathogen growth (Teixeira da Silva et al., 2017). They were then planted in pots containing media based on the *Trichoderma* spp. application treatment. During the first week of acclimatization, plastic covers were used to gradually adapt the plantlets to the ex-vitro temperature and humidity. On the fourth acclimatization day, the plastic cover was partially removed to expose the plantlets to lower humidity. The plants are maintained by watering daily according to the water capacity of the media.

Parameters and analysis. The parameters observed in this experiment were increment of plant height, stem diameter, leaf number, root quantity, root length, and leaf chlorophyll content index (CCI) of the acclimatized ramie seedlings for 4 weeks. CCI was measured using CCM-200 plus Chlorophyll Content Meter (Opti-Sciences Inc). The data obtained were analyzed using analysis of variance (ANOVA) and further analyzed using the least significant difference (LSD) at 5% probability.

Results and Discussion

The result in Table 1 indicates no interaction between plantlet age and *Trichoderma* spp. fertilizer application. However, each factor independently significantly influences all vegetative growth parameters of the plantlets. The increase in plant height is only influenced by the plantlet age. The initial plant height varies according to age, with average heights of 4, 8, and 12 weeks of age being 4.03, 10.65, and 16.14 cm, respectively. This is consistent with the study by Lommen (2023), which found that older in vitro plantlets had longer stems when transplanted than younger plantlets. They also had more main stem nodes, higher total dry weight, greater leaf area, and higher shoot and root weights.

The 4-week-old plantlets (a1) showed the best height increase compared to the other two older ages. This is because the 4-week-old plantlets still have young tissues, grow more actively, and have good vigor. A similar case occurred in the study by Sinta & Amanah (2019) that the stevia plantlets reached 10 cm in height but had low vigor, leading to difficulty standing upright when transplanted to multi-trays during hardening.

Their stem diameter also influenced the inability of some plantlets to stand upright. Older plantlets generally have larger stem diameters. After acclimatization, it was observed that the 4-week-old plantlets showed a greater increase in diameter compared to the 8-week-old plantlets, resulting in a larger diameter. Sulaiman et al. (2005) found higher survival rates in stem cuttings with larger diameters due to the influence of diameter on root number. This was evident in this experiment, where a greater increase in root quantity was observed in the 4-week-old plantlets, which also had a greater increase in diameter by the end of acclimatization. However, the 12-week-old plantlets still had the highest number of roots compared to the other age treatments. This was consistent with the study of Sinta & Amanah (2019), where the highest number and length of roots were found in the oldest plantlets. Besides the rooting induction media, some factors affecting root development are shoot age and size; younger shoots with stem diameters less than 0.4 cm produce roots more slowly (Karyanti et al., 2021; Lakho et al., 2023).

The longest root length was obtained from the 12-week-old plantlets, but the greatest increase in length was observed in the 4-week-old plantlets. Rooting is a critical factor in acclimatization, especially in nutrient uptake. Roots growing in agar-based media typically

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show fragility, often experience dysfunction, and lack root hairs. At the same time, transplanting is a vulnerable stage that leads to root and plantlet mortality after transfer to soil media (Erst et al., 2018). This occurred in the 8-week-old plantlets with underdeveloped roots, resulting in a lower survival rate.

In addition to root development, leaf number also plays a role in survival during acclimatization. The 4-week-old plantlets also showed the best growth parameters regarding stem diameter increase, leaf number, root number, and root length. The number of leaves for each plantlet age before acclimatization was the same, with 5 leaves for 4-week-old plantlets, 7 for 8-week-old plantlets, and 9 for 12-week-old plantlets. Based on the observations in Table 1, the highest increase in leaf number occurred in the 4-week-old plantlets, with an increase almost twice as large as the other two age treatments. This could be due to younger plantlets experiencing faster growth as young tissues are more active in cell division, and their cell walls are less complex than older plantlets (Duaja et al., 2020).

Palee et al. (2012) found that after being transplanted to the soil for 8 weeks, new leaves and roots formed in the surviving plantlets, but the in vitro-formed leaves became wilted or necrotic. This was also observed in this study, where older plantlets only experienced growth in the upper leaves, while the in vitro-formed lower leaves did not increase leaf area and tended to curl (Figure 1).

The treatment of *Trichoderma* spp. the three plantlet ages did not significantly affect the parameters of height increment, root quantity, root length, and leaf number (Table 1). Plant height, with or without *Trichoderma* spp., did not differ significantly among the ages. This is likely because *Trichoderma* spp. plays a role in organic matter decomposition and does not directly contribute to nutrient absorption for plant growth and development (Handini et al., 2020).

Regarding the parameters of leaf number increment and root quantity, the application of *Trichoderma* spp. fertilizer did not show a significant difference, but it still tended to increase both parameters. Similar results were obtained in the study by Syamsiyah et al. (2023), where adding *Trichoderma* biofertilizer combined with NPK fertilizer did not significantly affect the number of leaves in corn plants but tended to increase leaf number. *Trichoderma* can produce gibberellic acid (GA3), indoleacetic acid (IAA), and benzyl aminopurine (BAP) that can stimulate growth, such as leaf numbers when used in appropriate amounts (Sudantha & Suwardji, 2022).

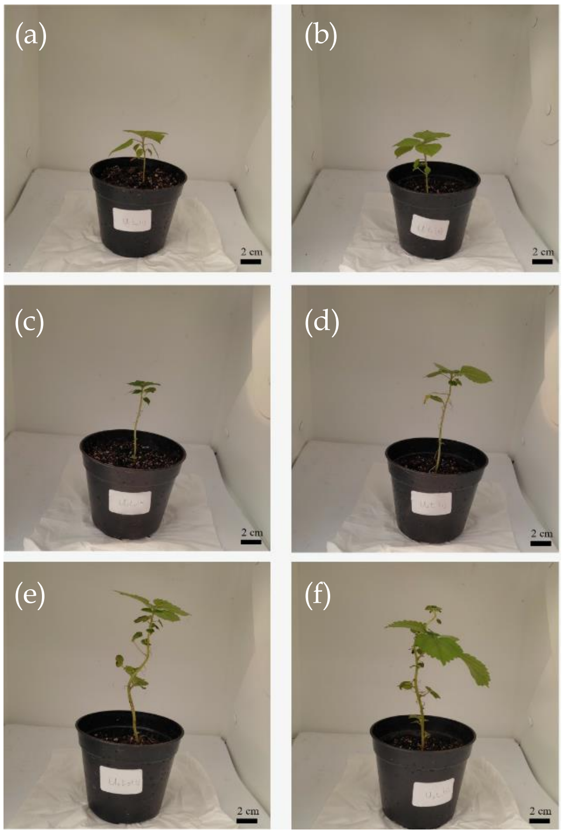

Figure 1. Visual appearance of ramie seedlings during acclimatization in each treatment: (a) a1t0; (b) a1t1; (c) a2t0; (d) a2t1; (e) a3t0; (f) a3t1.

The non-significant increase in leaf number could also be influenced by suboptimal root performance. Roots absorb nutrients from the media and transport them to other parts of the plant, including the leaves. In this study, it was observed that with *Trichoderma* spp. application, the root length tended to be lower than the control. A similar phenomenon was reported in Nieto-Jacobo et al. (2017), where Arabidopsis inoculated with *Trichoderma* in vitro showed a decrease of 13-25% in primary root length and an increase of 64-90% in secondary root quantity. It was found that the decrease in primary root length is a common phenomenon during *Trichoderma*-Arabidopsis interactions, potentially caused by reduced expression or localization of the auxin receptor PIN (PIN-FORMED Protein),

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which may be responsible for the reduction in root length (Nieto-Jacobo et al., 2017).

Unlike other growth parameters, the diameter increments of the stem showed a significant effect of *Trichoderma* spp. in increasing the stem diameter of the ramie seedlings (Table 1). The tendency of increased growth in ramie seedlings due to the application of *Trichoderma* spp. may contribute to the increased diameter. Similar results were observed in the study by Syamsiyah et al. (2023), where adding *Trichoderma* to NPK base fertilizer resulted in a larger stem diameter in corn plants compared to the control.

The age of the plantlet and the application of *Trichoderma* spp. show an interaction effect on the chlorophyll content index (CCI) parameter (Table 2). The chlorophyll content index increases with the age of the plantlets used. According to Tian et al. (2015), plants with hyperhydricity exhibit lower total chlorophyll content than non-hyperhydric shoots, which may be caused by the harmful effects of ROS (reactive oxygen species). Additionally, this condition can lead to changes in cellular redox homeostasis and chloroplast damage. This is also observed in this study, where younger plantlets experience hyperhydricity, resulting in lower chlorophyll content. According to Andrzejak & Janowska (2022), the decrease in chlorophyll index could be due to the application of *Trichoderma* spp., which leads to a decrease in leaf nitrogen balance index as a result of increased flavonoid content in the epidermis and a decrease in chlorophyll content, caused by a shift from primary to secondary metabolism. Despite the decrease in CCI in plantlets with *Trichoderma* spp. application, their growth is not disturbed. According to Liu et al. (2013), a reduction in pigments may not affect photosynthesis capacity; for example, ramie treated with gibberellin has lower chlorophyll a, b, and total content, even lower than those treated with drought, but it can produce higher fiber, stem length, and stem diameter. It was consistent with this study when 4-week-old plants with the lowest chlorophyll content can grow better than other ages.

Table 2. Interaction effect of Plantlet Age and *Trichoderma* spp. Application on leaf chlorophyll index

Chlorophyll content index (CCI)				
Plantlet ages	*Trichoderma* spp. application			
	t_0		t_1	
a_1	3.1250 a	C	2.6000 b	C
a_2	4.1250 a	B	3.8625 b	B
a_3	5.1500 a	A	4.4250 b	A

Explanation: The average values followed by the same letter are not significantly different according to the Least Significant Difference (LSD) test at the 0.05 significance level. Capital letters are read vertically to compare plantlet factors' age with the same *Trichoderma* spp. application. Small letters are read in the horizontal direction to compare the *Trichoderma* spp. application factors at the same plantlet age. a1: 4-week-old plantlets, a2: 8-week-old plantlets, a3: 12-week-old plantlets; t0: without *Trichoderma* spp. fertilizer application, t1: with *Trichoderma* spp. fertilizer application.

Table 1. Independent effect of plantlet age and *Trichoderma* spp. application on growth parameters.

Treatments	Plant Height Increment (cm)		Stem Diameter Increment (mm)		Roots Number Increment		Root Length Increment (cm)		Leaf Number Increment	
Plantlet ages(A)										
a_1	1.82	a	0.33	a	3.71	a	6.47	a	3.69	a
a_2	1.12	b	0.14	b	1.88	b	4.37	b	1.75	b
a_3	1.71	a	0.35	a	2.19	b	5.69	ab	1.88	b
LSD 5%	0.28		0.06		0.88		1.59		0.66	
Trichoderma spp. Application (T)										
t_0	1.66	a	0.23	b	2.51	a	5.64	a	2.42	a
t_1	1.44	a	0.32	a	2.67	a	5.38	a	2.46	a
LSD 5%	0.35		0.08		1.08		1.94		0.81	

Explanation: The values with the same letter notation do not show significant differences according to the Least Significant Difference (LSD) post-hoc test at the 5% significance level. a1: 4-week-old plantlets, a2: 8-week-old plantlets, a3: 12-week-old plantlets; t0: without *Trichoderma* spp. fertilizer application, t1: with *Trichoderma* spp. fertilizer application.

Conclusions

Based on the conducted research, the following conclusions can be drawn:

1. The 4-week-old plantlets showed the best growth during the acclimatization period of ramie seedlings, as evidenced by the significant increase in the height, the number of roots, the length of roots, and the number of leaves.
2. Applying *Trichoderma* spp. during the acclimatization period significantly increased the stem diameter of ramie seedlings, while other traits were not significantly affected by *Trichoderma* spp. application.

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References

Alexopoulus A, Petropoulus SA. 2021. Tissue Culture of Potato for Seed Production. In P. M. Villa. In P. M. Villa (Ed), The Potato Crop Management, Production, and Food Security (pp. 61-89). New York. Nova Science Publishers, Inc.

Andrzejak R, Janowska B. 2022. Flowering, nutritional status, and content of chloroplast pigments in leaves of *Gladiolus hybridus* L. 'Advances Red' after application of *Trichoderma* spp. Sustainability (Switzerland), 14(8).

Bag N, Palni LMS, Nandi SK. 2019. An efficient method for acclimatization: In vitro hardening of tissue culture-raised tea plants (*Camellia sinensis* (L.) O. Kuntze). Current Science, 117(2): 288–293.

Duaja M, Kartika E, Gusniwat G. 2020. Pembiakan Tanaman Secara Vegetatif. Jambi. Fakultas Ekonomi dan Bisnis Universitas Jambi Jalan.

Erst A, Gorbunov A, Karakulov A. 2018. Rooting and acclimatization of in vitro propagated microshoots of the *Ericaceae*. Journal of Applied Horticulture, 20(3): 177–181.

Handini AS., Rahhutami R, Astutik D. 2020. The effectiveness of humic acid and *Trichoderma* sp. for planting media with oil palm empty bunches in pakcoy (*Brassica rapa* L.). International Journal of Multi Discipline Science (IJ-MDS), 3(2): 44.

Hiti-Bandaralage JCA, Hayward A, Mitter N. 2017. Micropropagation of Avocado (*Persea Americana* Mill.). American Journal of Plant Sciences, 08(11), 2898–2921. 7

Karyanti, Khairiyah H, Sukarnih T, Hanifah NF, Rudiyana Y, Wahid A, Mira FR. 2021. In vitro induction of oil palm (*Elaeis guineensis* Jacq.) shoot roots and their acclimatization in mycorrhiza-enriched media. IOP Conference Series: Earth and Environmental Science, 759(1).

Lakho MA, Jatoi MA, Solangi N, Abul-Soad AA, Qazi MA, Abdi G. 2023. Optimizing in vitro nutrient and ex vitro soil mediums-driven responses for multiplication, rooting, and acclimatization of pineapple. Scientific Reports, 13(1): 1–10.

Liu T, Zhu S, Fu L, Yu Y, Tang Q, Tang S. 2013. Morphological and physiological changes of ramie (*Boehmeria nivea* L. Gaud) in response to drought stress and GA3 treatment1. Russian Journal of Plant Physiology, 60(6): 749–755.

Lommen WJM. 2023. Effects of age of in vitro-derived potato plantlets on early above- and below-ground development after planting in different cultivars. Potato Research.

Mukherjee PK, Mondal R, Dutta S, Meena K, Roy M, Mandal AB. 2018. In vitro micropropagation in *Boehmeria nivea* to generate safe planting materials for large-scale cultivation. Czech Journal of Genetics and Plant Breeding, 54(4): 183–189.

Muryanto M, Sudrajad P, Prasetyo A. 2018. Pengembangan tanaman rami (*Boehmeria nivea* L. Gaud) dan pemanfaatan limbah daun rami untuk penggemukkan domba wonosobo. Jurnal Litbang Provinsi Jawa Tengah, 16(2): 201–210.

Nieto-Jacobo MF, Steyaert JM, Salazar-Badillo FB, Vi Nguyen D, Rostás M, Braithwaite M, De Souza JT, Jimenez-Bremont JF, Ohkura M, Stewart A, Mendoza-Mendoza A. 2017. Environmental growth conditions of *Trichoderma* spp. Affects indole acetic acid derivatives, volatile organic compounds,

ISSN: 1412-4718, eISSN: 2581-138x

and plant growth promotion. Frontiers in Plant Science, 8.

Novarini E, Sukardan MD. 2015. Potensi serat rami (*Boehmeria nivea* S. Gaud) sebagai bahan baku industri tekstil dan produksi tekstil dan tekstil teknik. Arena Tekstil, 30(2): 113–122.

Palee J, Dheeranupattana S, Jatisatienr A, Wangkarn S, Mungkornasawakul P, Pyne S, Ung A, Sastraruji T. 2012. Influence of plantlet age and different soilless culture on acclimatization of *Stemona curtisii* Hook.f. Asian Journal of Plant Sciences, 11(6): 294–299.

Pirata MS, Correia S, Canhoto J. 2022. Ex vitro simultaneous acclimatization and rooting of in vitro propagated tamarillo plants (*Solanum betaceum* Cav.): Effect of the Substrate and Mineral Nutrition. Agronomy, 12(5).

Prausová R, Kozelková Z, Šafářová L. 2015. Protocol for acclimatization of in vitro cultured Potamogeton praelongus - Aspect of plantlet size and type of substrate. Acta Societatis Botanicorum Poloniae, 84(1): 35–41.

Purwati RD. 2010. Strategi pengembangan rami (*Boehmeria nivea* Gaud.). Perspektif, 9(2): 106–118.

Purwati RD, Sudjindro. 2004. Peluang perbanyakan bibit melalui kultur jaringan untuk mendukung pengembangan rami. In : Prosiding Lokakarya Model Pengembangan Agribisnis Rami, Garut 24 November 2007. Bogor : Puslitbangbun, 2007: P. 43-48, 43–48.

Saptari RT, Sinta MM, Budiani A. 2017. In vitro propagation of *Anthurium adreanum* cv. Nitta through organogenesis. Agrivita, 39(2): 192–200.

Sinta MM, Amanah DM. 2019. Acclimatization and early growth of tissue culture-derived *Stevia rebaudiana* at low altitude area in Bogor , Indonesia. Menara Perkebunan, 87(1): 68–76.

Sparta A, Emilda D. 2020. Growth evaluation of banana cv. Barangan as the effect of *Trichoderma* sp. and covering types during acclimatization process. Caraka Tani: Journal of Sustainable Agriculture, 35(2): 268.

Sudantha M, Suwardji. 2022. Growth response and yield of shallots to *Trichoderma* biostimulants and growth regulators substance benzyl amino purine (GRS BAP) . Proceedings of the 6th International Conference of Food, Agriculture, and Natural Resource (IC-FANRES 2021), 16: 289–297.

Suherman C, Nuraini A, Wulandari VSR. (2017). Respons tiga klon tanaman rami (Boehmeria nivea (L.) Gaud) terhadap konsentrasi asam giberelat yang berbeda. Kultivasi, 16(3): 494–501.

Sulaiman Z, Kemp PD, Douglas GB. 2005. Effects of stem diameter and planting depth on survival and early growth of field-planted willow and poplar. Agronomy New Zealand, Proceedings, 35(July 2016): 104–117.

Suparno O. 2020. Potensi dan masa depan serat alam indonesia sebagai bahan baku aneka industri. Jurnal Teknologi Industri Pertanian, 30(2): 221–227.

Syamsiyah J, Herdiansyah G, Hartati S. 2023. Use of *Trichoderma* as an Effort to increase growth and productivity of maize plants. IOP Conference Series: Earth and Environmental Science, 1165(1).

Teixeira da Silva JA, Hossain MM, Sharma M, Dobránszki J, Cardoso JC, Zeng S. 2017. Acclimatization of in vitro-derived dendrobium. Horticultural Plant Journal, 3(3): 110–124.

Tian J, Jiang F, Wu Z. 2015. The apoplastic oxidative burst as a key factor of hyperhydricity in garlic plantlet in vitro. Plant Cell, Tissue and Organ Culture, 120(2): 571–584.