



(*) Corresponding author:
etik.tini@unsoed.ac.id

Citation:

TINI E.W., ULINNUHA Z., KURNIAWAN R.R.,
WIDODO P., PRASETYO R., SUGIYONO, 2026 -
*Auxin-induced plantlet formation of Dendrobium
stratiotes x Dendrobium lineale hybrid in in vitro
culture*. - Adv. Hort. Sci., 40(3): 247-252.

ORCID:

TEW: 0000-0001-5122-5507
UZ: 0009-0003-8675-9252
KRR: 0009-0007-0174-7738
WP: 0000-0002-2203-7168
PR: 0000-0002-4683-9800
S: 0000-0003-2002-9196

Copyright:

© 2026 Tini E.W., Ulinnuha Z., Kurniawan R.R.,
Widodo P., Prasetyo R., Sugiyono. This is an open
access, peer reviewed article published by
Firenze University Press
(https://www.fupress.com) and distributed,
except where otherwise noted, under the terms
of CC BY 4.0 License for content and CC0 1.0
Universal for metadata.

Data Availability Statement:

All relevant data are within the paper and its
Supporting Information files.

Competing Interests:

The authors declare no conflict of interests.

Received for publication 15 January 2025

Accepted for publication 20 March 2026

Auxin-induced plantlet formation of *Dendrobium stratiotes* x *Dendrobium lineale* hybrid in *in vitro* culture

E.W. Tini ¹ (*), Z. Ulinnuha ¹, R.R. Kurniawan ¹, P. Widodo ², R. Prasetyo ³,
Sugiyono ²

¹ Faculty of Agriculture Jenderal Soedirman University, Jl. Dr. Soeparno
61 Purwokerto, 53122 Indonesia.

² Faculty of Biology Jenderal Soedirman University, Jl. dr. Soeparno 63
Purwokerto, 53122 Indonesia.

³ Faculty of Science and Technology, Riyadlul Ulum University, Tasikmalaya,
West Java, Indonesia.

Key words: Crossing, native species, orchid, plant growth regulators.

Abstract: Native species orchids, as a source of genetic diversity, can be used in crossing activities to produce hybrid orchids. *Dendrobium* is a type of orchid in great demand and of high economic value because of its unique flower shape and colour. Developing new varieties through interspecific crossings has produced a *D. stratiotes* × *D. lineale* hybrid that successfully germinated in *in vitro* culture. This experiment was carried out to study the effect of auxin type and concentration on *D. stratiotes* × *D. lineale* hybrid orchid plantlet development in *in vitro* culture, as well as to determine the best type and concentration of auxin to stimulate *D. stratiotes* × *D. lineale* hybrid orchid plantlet development. The results showed that the number of shoots formed and the root length of the *D. stratiotes* × *D. lineale* hybrid were controlled by the interaction between auxin type and its concentration. Adding 15 µM IBA produced the most shoots, 16.33 shoots per explant. In addition, NAA application resulted in the longest shoots (1.38 cm shoot per explants) and most roots (11 roots per explants) at 8 weeks after planting, which were significantly different from those produced by IAA or IBA.

1. Introduction

Orchids are included in the family Orchidaceae, which is widely distributed worldwide. Until now, Orchidaceae is estimated to encompass 700 to 800 genera and approximately 17,000 to 35,000 species worldwide (Widiarti *et al.*, 2023). A genus with a broad distribution is *Dendrobium*, which is one of the orchid genera widely cultivated for its high aesthetic and economic value and for its contribution to efforts to preserve biodiversity. This genus has many variations, including their habitat, shape, size, and flower colour (Apriliyani and Wahidah, 2021). *Dendrobium* is one type of orchid easily adapted to environmental stress, such as lack of water, high light intensity, and winter conditions,

compared to other orchids (Tikendra *et al.*, 2018). As a plant with many species that have not been identified in nature, orchids continue to be studied as a source of germplasm diversity.

Orchids have high aesthetic and economic value due to their uniqueness and rarity. The newly produced *D. stratiotes* × *D. lineale* variety has not yet been registered under the Plant Variety Protection (PVP) Act. PVP registration, which is «sui generis» of patents, protects plant varieties developed by plant breeders if they contain new, unique, uniform, and stable elements (Diah and Irianti, 2017). Tini *et al.* (2024) state that *Dendrobium stratiotes*, which originates from the Sunda Islands, Sulawesi, and Maluku (Halmahera and Morotai), flourishes in lowlands as epiphytes with medium-large sizes, enjoys warm to hot circumstances, and bunches together to form groups. *Dendrobium lineale* is a significant giant orchid species native to Papua that flourishes as an epiphyte or lithophyte in warm-to-hot climates. It is commonly found in riverbeds and coastal areas, up to 800 meters above sea level.

Growth regulators (PGRs) are among the factors that affect *in vitro* hybrid orchid propagation (Saad and Elshahed, 2012). PGRs play a part in controlling explant growth during both the plantlet production and multiplication stages. Auxin is one of the PGRs most commonly used for root induction and multiplication. Although auxin promotes cell division, expansion, and differentiation, its primary function is to induce an explant to form roots (Lavy and Estelle, 2016; Leyser, 2018). Auxins such as naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), and indole-3-butyric acid (IBA) are frequently utilized in root induction (Chandler, 2016; Prasetyo *et al.*, 2020; Shawky Soliman, 2020; Tamyiz *et al.*, 2022). This study aims to test the impact of auxin type and concentration on hybrid orchid progeny (*D. stratiotes* × *D. lineale*). Acclimatization will be more successful in offspring with a more muscular root system.

2. Materials and Methods

Plant materials

The plant materials utilized were microshoots of *D. stratiotes* × *D. lineale*, previously cultured on Murashige-Skoog (MS) medium (Phytotech M519) augmented with 10 µM BAP (Sigma-Aldrich B3408) and 30 g.L⁻¹ sucrose, solidified with 8% agar (SNP plant).

Root induction and plantlet formation

Plantlet development of *D. stratiotes* × *D. lineale* was performed on MS medium enriched with 30 g L⁻¹ sucrose and solidified with 8 g L⁻¹ agar. The effects of IAA (Sigma-Aldrich I2886), IBA (Sigma-Aldrich I5386), and NAA (Sigma-Aldrich N0640) at different concentrations were examined. The research was executed experimentally, employing a split-plot design with three replications. The primary plots represented auxin types, specifically IAA, IBA, and NAA, while the secondary plots indicated auxin concentrations of 0 µM, 5 µM, 10 µM, and 15 µM. The medium was calibrated to pH 5.8. The microshoots were cultivated in a single explant per bottle and incubated at 24°C under continuous illumination for 8 weeks. The observed variable was the production of *D. stratiotes* × *D. lineale* plantlets, with the measured parameters being the number of shoots, leaves, roots, and plant length.

Data analysis

The data were analyzed using analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) at a 95% confidence level using DSASTAT VER 1.514 software.

3. Results

According to the observation results, the hybrid orchid explants *D. stratiotes* × *D. lineale* showed significant development, as evidenced by increased shoot number, leaf number, shoot length, root number, and root length in each treatment. The summary of the analysis of variance (ANOVA) presented in Table 1 shows that the types of auxins significantly affect the shoot length and the number of leaves. Auxin concentration significantly affects the number of shoots but does not significantly affect the number of leaves, shoot length, roots, or root length. Furthermore, the interaction between auxin type and concentration substantially affects the number of shoots and roots. However, it does not significantly affect leaf number, shoot length, or root number.

One of the critical parameters in the formation of *D. stratiotes* × *D. lineale* plantlets is the growth of axillary shoots. The analysis of variance (ANOVA) (Table 1) shows that the number of shoots formed is influenced by the concentration of auxin and the interaction between the type and concentration of auxin. The DMRT results (Fig. 1 and 2) show that the

average number of shoots is highest at a concentration of 15 μM , with 12 shoots per explant. This result is significantly different from all the tested auxin concentrations. The data in figure 1 shows that an increase in auxin concentration led to an increase in the number of shoots formed. The lowest average number of shoots was observed at a concentration of 5 μM , with 4.22 shoots per explant.

Interestingly, the control treatment (0 μM) has a higher average number of shoots per explant (5.11) than the 5 μM treatment. The increase in the number of shoots in media without PGR indicates a high cytokinin content in the explants, which can be caused by using BAP during the multiplication stages. BAP is a cytokinin that can bind to sugar and form a BAP-sugar bond that can be stored longer in plants. The results of this study differ from those of Tikendra *et al.* (2018), who reported that a 10 μM auxin concentration induced the best shoot and root formation in *D. thyrsiflorum* Rchb.f cultures, with an

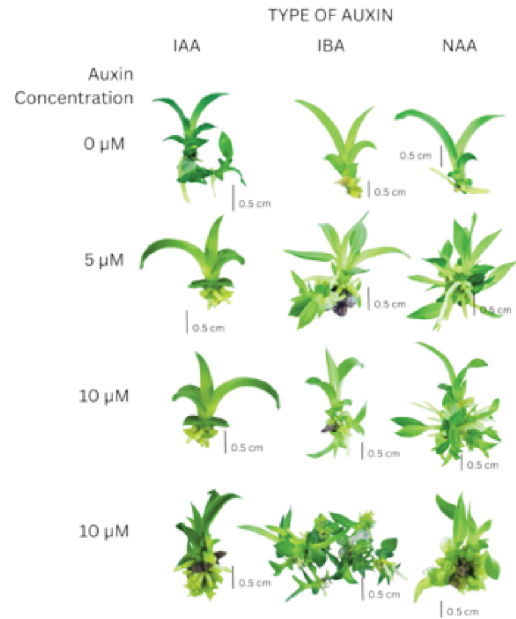


Fig. 2 - The appearance of *D. stratiotes* x *D. lineale* plantlets on media with different types and concentrations of auxin at 8 weeks after planting.

Table 1 - Summary of the analysis of variance (ANOVA) results on the effect of auxin types and concentrations on the parameters of *D. stratiotes* x *D. lineale* hybrid orchid plantlet formation

Source of variance	p value of				
	No. of shoots	No. of leaves	Shoot height	No. of Root	Root length
Auxin type (A)	0.951	0.840	0.040	0.011	0.607
Auxin concentration (K)	0.015	0.291	0.098	0.670	0.050
Interaction A x K	0.046	0.198	0.112	0.332	0.013

p-value <0,01 shows a very significant difference at a 1 % error rate of F-test; p-value < 0,05 shows a very significant difference at 5% error rate of F-test.

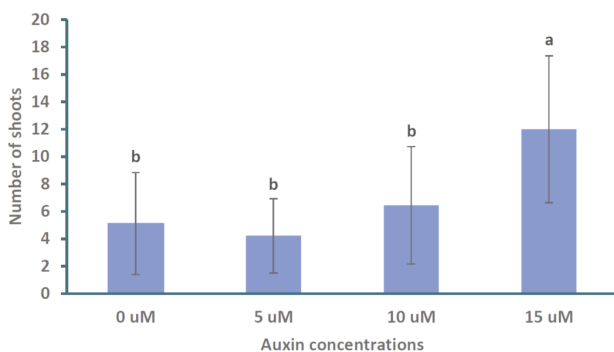


Fig. 1 - The results of DMRT on the average number of shoots of *D. stratiotes* x *D. lineale* hybrid as a response to auxin concentration at 8 weeks after planting. Means marked with different letters are statistically different at DMRT 95%.

average of 3.04 shoots per explant and 6.52 roots per explant. Furthermore, Suryani and Ratnawati (2024) found that the use of auxin (NAA) at a low concentration (0.2 ppm = 1.07 μM) was able to

induce the most *Dendrobium* sp. shoots, with an average of 1.44 shoots per explant.

The DMRT test results on the effect of the interaction between auxin types and concentrations on *D. stratiotes* x *D. lineale* plantlet formation (Fig. 3) showed that the 15 μM IBA treatment produced the highest number of shoots, i.e., 16.33 shoots per explant. However, it was not significantly different from those produced by 15 μM IAA and 10 μM NAA, with average shoot counts of 13.67 and 11.33 per explants, respectively. According to Sauer *et al.* (2013), high auxin concentrations can synergize with endogenous cytokinins to increase the number of shoots in culture. Furthermore, Prasetyo *et al.* (2020) state that using the appropriate concentration can accelerate the cell cycle, thereby speeding cell division and differentiation. Barua *et al.* (2022) stated that using 1.5 mg L⁻¹ IBA (8.055 μM) supplemented with 0.2% banana extract induced the number of

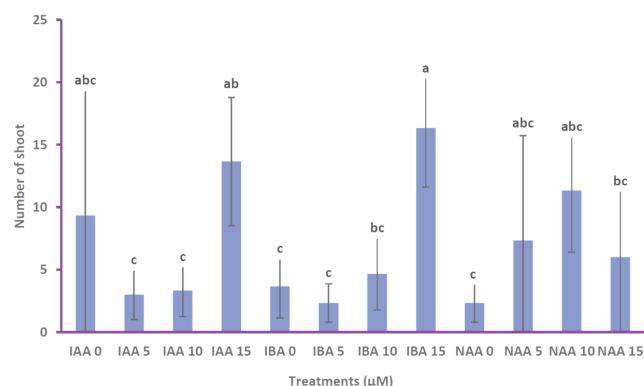


Fig. 3 - The results of DMRT on the average number of shoots of *D. stratiotes* × *D. lineale* hybrid as a response to the interaction between auxin types and concentrations at 8 weeks after planting. Means marked with different letters are statistically different at DMRT 95%.

shoots of *Dendrobium lituiflorum* Lindl. with an average of 14.5 shoots per explant.

Figure 3 shows that the lowest average number of shoots was produced by 0 µM NAA (without PGR/control) and five µM IBA (2.33 shoots per explant). The results of this observation indicate that the addition of auxin to the media can increase the induction of lateral shoots in the hybrid orchid *D. stratiotes* × *D. lineale*. Based on Dobrev *et al.* (2017), auxin can synergize with cytokinin in the formation of axillary shoots by accelerating mitosis.

According to Poonthong *et al.* (2018), optimizing nutrient levels in culture media can lead to substantial changes in shoot morphology, including increased shoot length and a greater number of larger, healthier leaves. Putri *et al.* (2024) stated that MS media contains elevated levels of mineral salts and is suitable for most plants, hence optimizing explant growth. According to Müller and Leyser (2011), a high concentration of auxin in the explant caused by exogenous auxin application stimulates ethylene synthesis, thereby inhibiting cell elongation. However, if exogenous auxin is insufficient, growth is suboptimal.

The DMRT result on the effect of auxin types on average shoot length (Fig. 4) showed that NAA yielded the highest average shoot length (1.38 cm). The results obtained differ significantly from those of every other auxin type examined. The media supplemented with IBA showed the shortest shoot length (1.01 cm), followed by the media supplemented with IAA (1.04 cm). All three auxin types tested increased plant length, indicating auxin's role in controlling elongation in explants. Kumar *et al.* (2019) and Saini *et al.* (2013) state that auxin is crucial

for cell elongation and enlargement, cell differentiation, and root induction. NAA is a kind of auxin that effectively stimulates root development within a limited concentration range. Tolera (2016) found that NAA and IBA are auxins commonly utilized in the vegetative propagation of sugarcane due to their significant rhizogenic capacity and stability in plant tissue. Furthermore, Kazan (2013) and Pandey *et al.* (2019) state that the auxin hormone signaling stretches cell walls, thereby promoting cell elongation.

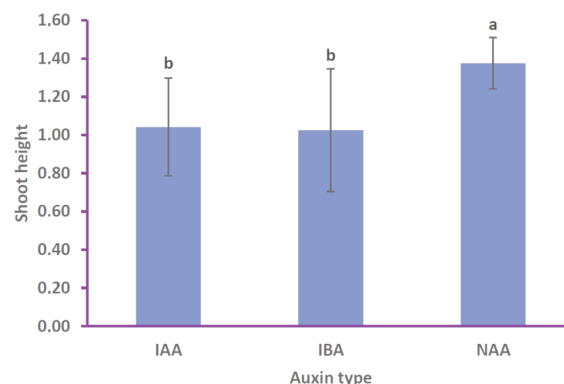


Fig. 4 - The results of DMRT on the average shoot length of *D. stratiotes* × *D. lineale* hybrid as a response to auxin types at 8 weeks after planting. Means marked with different letters are statistically different at DMRT 95%.

Auxin promotes elongation by activating a proton pump at the plasma membrane, secreting H⁺ ions into the cell wall, thereby lowering pH and facilitating the loosening of cellulose fibers by disrupting their interconnections. Moreover, auxin enhances the expression of expansins, thereby increasing the elasticity of the cell wall. As the cell wall becomes more pliable, an input of water (to be sequestered in the vacuole) results in cellular enlargement (Blakeslee *et al.*, 2005; Zhao, 2014; Leyser, 2018).

One of the important parameters in the formation of *D. stratiotes* × *D. lineale* plantlets is the growth of roots. Table 1 shows that the type of auxin treatment influences root formation. The DMRT result on the effect of auxin types on the average number of roots (Fig. 5) showed that NAA yielded the highest number of roots (11.00 roots per explant). However, this result was similar to the IBA treatment, which had an average of 8.33 roots per explant. In contrast, the lowest average number of roots was observed in the IAA-supplemented media, namely 4.33 roots per explant. According to Arli and Noli (2023), NAA 1.5 mg L⁻¹ is the best concentration for root formation in *Dendrobium lasianthera* orchids. Furthermore, Lamboro *et al.* (2022) found that 4 mg L⁻¹ NAA

induced the greatest rooting in *Arachis hypogaea* cv. Chinese peanut cultivar N3 and Yu-Hua-14 cultures, with an average of 10 roots per explant.

Based on the results of DMRT, the effect of interaction between the type and concentration of auxin on the root length of *D. stratiotes* x *D. lineale* at the age of 8 WAP (Fig. 6) showed that the media supplemented with 10 μM NAA can induce the longest root length with an average of 1.93 cm. The 10 μM NAA treatment showed the best results compared to the media supplemented with IAA and IBA at the same concentration. These results differ from those of Prasetyo *et al.* (2020), who reported that using NAA in sugarcane culture was ineffective at inducing root length; the short root length supports this finding. NAA is included in synthetic auxins used to initiate root formation in plants (Nugroho, 2023). The results of this study differ from those of Bhowmik and Rahman (2020), who stated that *Dendrobium palpebrae* Lindl. cultured in auxin-free media induced the longest roots, averaging 4.82 cm per explant.

Furthermore, Mallik *et al.* (2015) cultured hybrid *Dendrobium* orchids on media supplemented with 1 mg L^{-1} IAA and 1 mg L^{-1} IBA, which induced a root length of 2.29 cm. The data obtained showed that the shortest average root length was observed in media supplemented with 5 μM IAA. Based on the average root length data obtained, it is assumed that using the right concentration can induce good growth in the formation of hybrid orchid expanses of *D. stratiotes* x *D. lineale* hybrid.

The treatment of auxin types and concentrations on *D. stratiotes* x *D. lineale* hybrid showed that the number of shoots formed and the root length were

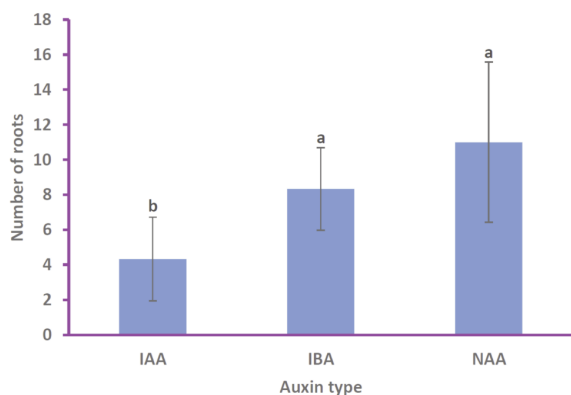


Fig. 5 - The results of DMRT on the average number of roots of *D. stratiotes* x *D. lineale* hybrid as a response to auxin types at 8 weeks after planting. Means marked with different letters are statistically different at DMRT 95%.

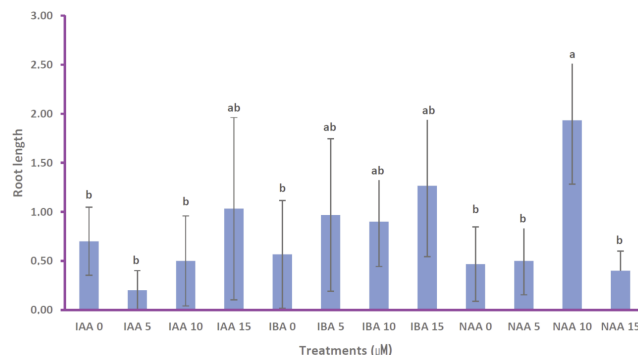


Fig. 6 - The results of DMRT on the average root length of *D. stratiotes* x *D. lineale* hybrid as a response to the interaction between auxin types and concentrations at 8 weeks after planting. Means marked with different letters are statistically different at DMRT 95%.

controlled by the interaction between auxin type and its concentration. The addition of 15 μM IBA produced the most shoots, 16.33 shoots per explant. In addition, NAA application resulted in the longest shoots (1.38 cm shoot per explant) and most roots (11 roots per explant) at 8 weeks after planting, which were significantly different from those produced by IAA or IBA.

Acknowledgements

The authors would like to express their sincere gratitude to the Research and Community Service Institute of Jenderal Soedirman University, Purwokerto, Indonesia, for providing the facilities and resources necessary for this research, as written in the contract Pelaksanaan Program Penelitian Riset Dasar Unseod Lanjutan Tahun Anggaran 2024 No: 26.408/UN23.35.5/PT.01/II/2024.

References

- APRILIYANI R., WAHIDAH B.F., 2021 - *Perbanyakan anggrek Dendrobium sp. secara in vitro: Faktor-faktor keberhasilannya*. - Filogeni: Jurnal Mahasiswa Biologi, 1(2): 33-36.
- ARLI N., NOLI Z.A., 2023 - *Induksi Akar Anggrek Dendrobium lasianthera dengan Pemberian Beberapa Konsentrasi Naphthalene Acetic Acid (NAA) Secara in vitro*. - Bioscientist: Jurnal Ilmiah Biologi, 11(2): 1369-1375.
- BARUA K.N., SINGHA B.L., BORA B., BORDOLOI S., 2022 - *In vitro seed propagation of endangered Dendrobium lituiflorum Lindl. and D. aduncum Lindl.* - Int. J. Pharm. Biol. Sci. Arch., 3: 108-115.

- BHOWMIK T.K., RAHMAN M.M., 2020 - *Micropropagation of commercially important orchid Dendrobium palpebrae lindl. through in vitro developed pseudobulb culture.* - J. Adv. Biotech. Exp. Therapeutics, 3(3): 225-232.
- BLAKESLEE J.J., PEER W.A., MURPHY A.S., 2005 - *Auxin transport.* - Curr. Opin. Plant Biol., 8(5): 494-500.
- CHANDLER J.W., 2016 - *Auxin response factors.* - Plant Cell Envir., 39(5): 1014-1028.
- DIAH Y.D.W.S.I., IRIANTI W.S., 2017 - *Perjanjian benefit sharing sebagai upaya perlindungan dan pemanfaatan varietas tanaman.* - Rechtidee, 12(1): 1-26.
- DOBREV P.I., HOYEROVÁ K., PETRÁŠEK J., 2017 - *Analytical determination of auxins and cytokinins*, pp. 31-39. - In: DANDEKART T., and M. NASEEM (eds.) *Auxins and cytokinins in plant biology: Methods and protocols.* Springer protocols. Humana Press, New York, NY, USA, pp. 204.
- KAZAN K., 2013 - *Auxin and the integration of environmental signals into plant root development.* - Annals Bot., 112(9): 1655-1665.
- KUMAR S., MALIK A., YADAV R., YADAV G., 2019 - *Role of different rooting media and auxins for rooting in floricultural crops: A review.* - Int. J. Chem. Studies, 7(2): 1778-1783.
- LAMBORO A., HAN X., YANG S.N., LI X.Y., YAO D., MOUSSA A.A., CHAUDHRY M.R., ILBOUDO H., SONG B., WU Q., XING Y., ZHANG J., 2022 - *High frequency direct organogenesis from cotyledonary node explants and plantlet regeneration of peanut (Arachis hypogaea) cultivars.* - Int. J. Agric. Biol., 27: 105-114.
- LAVY M., ESTELLE M., 2016 - *Mechanisms of auxin signaling.* - Development (Cambridge), 143(18): 3226-3229.
- LEYSER O., 2018 - *Auxin signaling.* - Plant Physiol., 176(1): 465-479.
- MALLIK R., MALI S.K., DEBNATH S., KHAN A.B.M.M.M., MALLIK M.R., MALI S.K., ISLAM M.R., HAQUE M.R., 2015 - *In vitro root formation of Dendrobium orchid plantlets with the influence of different growth regulators.* - J. Bangladesh Soc. Agric. Sci. Technol, 12(4): 71-74.
- MÜLLER D., LEYSER O., 2011 - *Auxin, cytokinin and the control of shoot branching.* - Annals Bot., 107(7): 1203-1212.
- NUGROHO D., 2023 - *Application of photosynthetic bacteria and various NAA (naphthalene 1 acetic acid) concentration on the growth of vanilla cuttings (Vanilla planifolia Andrews.).* - Indones. J. Interdiscip. Res. Sci. Technol., 1(9): 767-780.
- PANDEY V., BHATT I.D., NANDI S.K., 2019 - *Role and regulation of auxin signaling in abiotic stress tolerance*, pp. 319-331. - In: KHAN M.I.R., A. FERRANTE and N.A. KHAN (eds.) *Plant signaling molecules: Role and regulation under stressful environments.* Elsevier, Woodhead Publishing, Cambridge, UK, pp. 581.
- POOTHONG S., KHEN T., CHUMPHUKAM O., 2018 - *In vitro mineral nutrition for improving growth and multiplication of stevia.* - Agric. Nat. Resources, 52(5): 477-483.
- PRASETYO R., SUGIYONO, PROKLAMASININGSIH E., DEWI P.S., 2020 - *Plantlet formation and acclimatization of sugarcane cv. PS 881 with different types and concentration of auxin.* Biosaintifik, 12(3): 453-458.
- PUTRI A.K., PRASETYO R., PROKLAMASININGSIH E., DAVISON P.A., SUGIYONO S., 2024 - *Modification of media formulation and agar concentration to improve pitcher plant (Nepenthes mirabilis (Lour.) Druce) micropropagation for conservation and microfloriculture development.* - AgriHealth: J. Agri-Food, Nutrition Public Health, 5(1): 41.
- SAAD A.I.M., ELSHAHED A.M., 2012 - *Plant tissue culture media*, pp. 29-40. - In: LEVA A., and L.M.R. RINALDI (eds.) *Recent advances in plant in vitro culture.* Intech Open, London, UK, pp. 222.
- SAINI S., SHARMA I., KAUR N., PATI P.K., 2013 - *Auxin: A master regulator in plant root development.* - Plant Cell Reports, 32(6): 741-757.
- SAUER M., ROBERT S., KLEINE-VEHN J., 2013 - *Auxin: Simply complicated.* - J. Exp. Bot., 64(9): 2565-2577.
- SHAWKY SOLIMAN A., 2020 - *Plant growth hormones*, pp. 1-11. - In: VIKAS B., and M. FASULLO (eds.) *Cell Growth.* Intech Open, London, UK, pp. 128.
- SURYANI S., RATNAWATI R., 2024 - *Pengaruh kombinasi konsentrasi zat pengatur tumbuh naa dan 2-ip terhadap pertumbuhan anggrek Dendrobium sp. Secara in vitro.* - Kingdom J. Biol. Studies, 10(1): 14-33.
- TAMYIZ M., PRAYOGA L., PRASETYO R., MURCHIE E.H., SUGIYONO S., 2022 - *Improving agarwood (Aquilaria malaccensis Lamk.) Plantlet formation using various types and concentrations of auxins.* - Caraka Tani: J. Sust. Agric., 37(1): 142-151.
- TIKENDRA L., AMOM T., NONGDAM P., 2018 - *Effect of phytohormones on rapid in vitro propagation of Dendrobium Thyrsiflorum Rchb. f.: An endangered medicinal orchid.* - Pharmacognosy Magazine, 14(58): 495.
- TINI E., WIDODO P., SUGIYONO, ULINNUHA Z., 2024 - *Assessment of genetic parameters and heritability of Dendrobium species section Spatulata native to Indonesia.* - Adv. Hort. Sci., 38(3): 239-248.
- TOLERA B., 2016 - *Effects of naphthalene acetic acid (NAA) and indole 3 butyric acid (IBA) on in vitro rooting of sugarcane (Saccharum officinarum L.) micro shoots.* - J. Biotechnol. Biomater., 6(1): 215-221.
- WIDIARTI W., USMAN M., WAMILIANA W., NURCAHYANI N., MASTER J., 2023 - *Konservasi Anggrek Dan Peningkatan Peringkat Greenmetric Melalui Kegiatan Penanaman Anggrek Di Kampus.* - J. Pengabdian Masyarakat Tapis Berseri, 2(1).
- ZHAO Y., 2014 - *Auxin Biosynthesis.* - Arabidopsis Book, 12(12): e0173.