Buletin Kebun Raya 24(2): 51–56, Agustus 2021

e-*ISSN*: 2460-1519
p-ISSN: 0125-961X

https://publikasikr.lipi.go.id/index.php/buletin

Buletin Kebun Raya

The Botanic Gardens Bulletin

Scientific Article

A PRELIMINARY STUDY OF *IN VIVO* INJECTION OF AUXIN AND CYTOKININ INTO *Rafflesia patma* Blume FLOWER BUDS

Studi awal injeksi in vivo auksin dan sitokinin pada kuncup bunga Rafflesia patma Blume

Sofi Mursidawati[1*], Adhityo Wicaksono[2]

[1]Research Center for Plant Conservation and Botanic Gardens–Indonesian Institute of Sciences (LIPI)
Jl. Ir. H. Juanda No.13, Bogor 16003, Indonesia
[2]Division of Biotechnology, Generasi Biologi Indonesia Foundation
Jl. Swadaya Barat No. 4, Gresik 61171, Indonesia

Informasi Artikel

Diterima/Received : 4 Januari 2021
Disetujui/Accepted : 19 Juni 2021
Diterbitkan/Published : 1 Agustus 2021

***Koresponden E-mail** :
sofi.mdawati@gmail.com
adhityo.wicaksono@gmail.com

DOI: https://doi.org/10.14203/bkr.v24i2.670

Cara mengutip :
Mursidawati S, Wicaksono A. 2021. A preliminary study of in vivo injection of auxin and cytokinin into *Rafflesia patma* Blume flower buds. Buletin Kebun Raya 24(2): 52–56.
DOI: https://doi.org/10.14203/bkr.v24i2.670

Kontributor

Kontributor Utama/Main author:
Sofi Mursidawati
Adhityo Wicaksono

Kontributor Anggota/Author member:
-

Kata Kunci: fitohormon, holoparasit, perkembangan bunga, Rafflesiaceae, tumbuhan parasit
Keywords: flower development, holoparasite, parasitic plant, phytohormones, Rafflesiaceae

Abstrak

Mekanisme yang mengendalikan pertumbuhan dan diferensiasi *Rafflesia* dari kuncup bunga hingga tahap anthesis belum diketahui sampai saat ini, terutama peran zat pengatur tumbuh (ZPT) dalam mekanisme fisiologinya. Jumlah kuncup bunga tanaman ini di alam yang tersedia untuk penelitian sangat terbatas. Studi ini menggunakan enam kuncup bunga *Rafflesia patma* Blume yang dikelompokkan dalam tiga perlakuan berbeda, yaitu dua kuncup diinjeksi dengan auksin (asam indolasetat-IAA), dua kuncup diinjeksi oleh sitokinin (kinetin), dan dua kuncup terakhir diinjeksi oleh akuades steril sebagai kontrol negatif. Hasil penelitian menunjukkan adanya perbesaran kuncup pada perlakuan IAA dan kinetin dibandingkan kontrol, tetapi hanya pada perlakuan IAA yang menunjukkan tahap transisi dengan merekahnya braktea (tahap kupula ketahap kupula braktea) dalam dua minggu dari lima minggu lama pengamatan. Hal ini memunculkan pertanyaan apakah perkembangan *Rafflesia* lebih bergantung pada auksin dibandingkan sitokinin. Rekomendasi untuk penelitian selanjutnya adalah penggunaan sampel kuncup yang lebih banyak, penerapan cara pemberian hormon dengan lebih baik, penggunaan ZPT lain seperti giberelin (GA) dan asam jasmonat (JA), dan penerapan analisis histologi untuk melihat pengaruh ZPT yang diberikan pada perkembangan jaringan pada kuncup.

Abstract

The controlling mechanisms for the growth and differentiation of *Rafflesia* from a flower bud into the anthesis stage is currently unknown, particularly if any plant growth regulator (PGR) physiological pathways play some type of roles. In the wild, the number of flower buds available to study are extremely limited. In this study, we grouped six flower buds of *Rafflesia patma* Blume into three different treatments: two buds injected with auxin (indoleacetic acid, IAA), two buds injected with cytokinin (kinetin), and two buds injected with sterile distilled water as a control. Buds enlarged with both IAA and kinetin treatments compared to the control, but only buds injected with IAA showed a transition stage with the bract revealed (cupule-bract stage from previously cupule stage) within two weeks of five weeks of observation. These results raise further questions whether *Rafflesia* development is more likely due to auxin exposure when in flower bud as compared to cytokinin. Future studies should include increased sample size for treatments, enhanced PGR administration to allow exposure to the tissue and less tissue damage, injection of other PGRs such as gibberellin (GA) and jasmonic acid (JA), and histological tissue analysis to investigate PGR effects in depth.

INTRODUCTION

Researchers have tried to understand the mechanism that leads to *Rafflesia* anthesis for many years. Unlike any other endoholoparasitic plant (i.e. holoparasitic plant with its tissue embedded into the host plant), Rafflesiaceae has fragmented endophytes scattered inside the host body, *Tetrastigma* (Vitaceae) (Nikolov *et al*. 2014; Mursidawati *et al*. 2019). These scattered parasitic tissues hypothetically come from one germinating point and spread into the whole host plant tissue (Wicaksono *et al*. 2017), most probably as clones (Wicaksono *et al*. 2020). The parasitic endophytic cells proliferate by the push of the forces from the surrounding dividing cells of its host and these actively dividing cells reside within the vascular cambium tissue (Mursidawati *et al*. 2019). Alternatively, it has been suggested that the surrounding root new cortex tissue in the *Tetrastigma* might also contribute to the endophyte "growth vessel". This suggestion comes as the new cortex or the pericycle tissue replaced the original cortex tissue when the secondary root is developed as observed in another Vitaceae, *Vitis* sp. (Gambetta *et al*. 2013). However, between the scattered endophytes, no study so far has unraveled the physiological and molecular signaling between the endophyte clones, which might trigger the anthesis stage of the bud.

There are several plant growth regulators (PGR) that contribute to flower development, like auxin and cytokinin. Auxin as observed in *Arabidopsis thaliana* (L.) Heynh. (Alabadí *et al*. 2009) and in *Camelia azalea* C.F. Wei (Fan *et al*. 2015) initiates primordial flower growth. Cytokinin triggers the growth of flower organs, e.g. flower bud and ovule formation, and regulates flower and seed size (van der Krieken 1989; Bartrina *et al*. 2011). The effect of these regulators on the growth of *Rafflesia cantleyi* Solms-Laubach was observed in a transcriptomic study of genes and transcription factors related to auxin, cytokinin, gibberellin, abscisic acid, and jasmonic acid (Amini *et al*. 2019). As *Rafflesia* endophyte growth is cryptic, observations on the effects of PGR on flower growth can only be done via two possible ways, i.e. culture of the *Rafflesia* tissue and *in vivo* manipulation.

While several studies have been conducted on culturing *Rafflesia* tissue (Sukamto 2001; Mursidawati & Handini 2009; Sukamto & Mujiono 2010; Wicaksono & Teixeira da Silva 2015; Molina *et al*. 2017), only one has successfully induced the cultured bud tissue of *R. arnoldii* R.Br into callus tissues (Sukamto & Mujiono 2010). The callus tissues proliferated into the whitish strands under the picloram (synthetic auxin) treatments, but no response was observed on the zeatin (cytokinin) (Sukamto & Mujiono 2010). The strands might be consistent with Nikolov *et al*. (2014) uniseriate strands of *Rafflesia* in the early stage of endophyte growth inside the host tissue, which later may form cellular clusters before differentiating into a flower bud (Mursidawati *et al*. 2019; Wicaksono *et al*. 2020). From the results in Sukamto & Mujiono (2010) so far, this *in vitro* study method could offer an effective way to visually observe the vegetative growth of the endophytic tissue because no other study has successfully managed to differentiate the *Rafflesia* callus into a flower bud. On the later stage of flower development, the generative stage, an *in vivo* study performed in this short study might reveal the effect of the PGR on the growing flower bud of *Rafflesia*.

This study was an attempt to replicate one method from Mariani *et al*. (2011) in *Aglaonema* on *R. patma* Blume, although the procedure was performed in a non-analogous organ (*Aglaonema* stem vs. *Rafflesia* flower bud in Mariani *et al*. (2011) vs. this study, respectively). The study on *Aglaonema* involved an injection of benzyladenopurine (BAP) (synthetic cytokinin) into the axillary node of the stem to induce the axillary bud growth. For the present study, indoleacetic acid (IAA) (auxin) and kinetin (cytokinin) were used. Hypothetically, based on Sukamto & Mujiono (2010), the auxin could induce bud growth better than cytokinin. However, it was unclear if any other effects could be rendered since the whole flower bud organ was involved instead of only an undifferentiated callus. Nevertheless, this study is the first preliminary attempt of *in vivo* PGR induction on *Rafflesia*.

MATERIALS AND METHODS

Plant materials

The *Rafflesia patma* plant grown on its host plant *Tetrastigma leucostaphylum* (Dennst.) Alston ex Mabb was a result of grafting between the grown host vine rootstock in Bogor Botanical Garden and host vine scion from its natural habitat in Pangandaran Nature Reserve, West Java, Indonesia, as described in Mursidawati *et al*. (2015). This same plant was also used in previous studies (Wicaksono *et al*. 2017; Mursidawati *et al*. 2019; Mursidawati *et al*. 2020; Wicaksono & Mursidawati 2020). The experiment started on July 28, 2020 on six selected flower buds in cupule stage (Susatya 2020), approximately 5–6 months old on *T. leucostaphylum* roots. The number of buds was limited due to their availability at the time of the experiment at Bogor Botanical Garden.

Injection and observation

The injections followed the *Aglaonema* protocol by Mariani *et al*. (2011). Six flower buds were used for the present study, with two buds per treatment/control. Plastic labels were attached to the nearby root to identify the bud and the type of treatment or control it received. Every bud within the same treatment/control were on separate *T. leucostaphylum* roots to prevent any possibilities of cross-contamination effects between treatments or between treatment and control. All injections used three different short-needle sterile syringes (insulin syringe) per treatment with a maximum volume of 100 ml. The first treatment was 3 mg/l IAA, the second was 1 mg/l kinetin, and the third was sterile distilled water as the control. According to Mariani *et al*. (2011), the injected cytokinin was 30 mg/l and the tissue culture auxin and cytokinin was 3 mg/l for both. However, we used the low concentrations used in the tissue culture to prevent any shock to the tissue, as high cytokinin level may lead to hypersensitivity response similar to the condition during pathogenic attack by killing the cells at the infected site (Novák *et al*. 2013). In similar manner, high auxin concentration possesses herbicidal effect

(Grossmann 2007). To make the stock, 3 mg of IAA powder was first dissolved with 1 ml of NaOH (0.2 M) to dissolve it before being added to the 100 ml of sterile distilled water. Similarly, 3 mg of kinetin powder was first dissolved with 1 ml of NaOH (0.2 M) before being added to the 100 ml of sterile distilled water. In the end, 30 mg/l of each IAA and kinetin were acquired as the stocks. To obtain final 3 mg/l concentration, each stock was diluted 10 times using sterile distilled water.

The syringe needle was administered at the base (proximal) closest to *Tetrastigma* of flower bud cupule to avoid damaging the growing flower bud, especially since the *R. patma* bud meristematic tissue growth is more active at the distal region while the proximal region already has matured parenchyma cells (Mursidawati & Wicaksono 2020). The needle only pierced the bud approximately halfway the length of the whole needle (i.e. the needle is 1 cm long, only 0.5 cm is pierced) to minimize the damage to the bud and subsequent stress responses. Upon injection, the *R. patma* flower bud was very robust, so the injection was performed to be as slow as possible to maximize the possible intake of PGR (and water) by the buds, despite any drawback leaks caused by the resisting hard tissue of the flower bud. The injected buds were measured at the start of the experiment and then every week using Vernier caliper until stagnant growth was reached or death occurred. The growth data were averaged and divided per unit of time (week) to obtain the difference in growth rate (cm/week) per treatment/control.

RESULTS AND DISCUSSIONS

General growth pattern

From the initial week (week 0) of the experiment, the buds appeared to grow before they entered stagnation on the third week (Table 1). The stagnation conditions across kinetin and IAA treatments might indicate that the PGR effects only last for two weeks. Our results were similar to Mariani *et al*. (2011) which showed stagnation of axillary growth within two weeks. Compared to the control, bud growth was slow, observed towards the first week, and from the third week towards the fourth week.

Table 1. Flower bud measurements of *R. patma* per variable.

Week	Bud Diameter (cm)					
	Kinetin		IAA		Control	
	K1	K2	K3	K4	K5	K6
0	2.07	2.50	2.77	3.75	2.15	2.15
1	2.08	2.90	2.78	3.80	2.19	2.19
2	2.18	3.20	3.10	3.84*	2.19	2.19
3	2.18	3.28	3.30	3.90*	2.19	2.19
4	2.20	3.30	3.34	3.90*	2.21	2.22
5	2.20	3.30	3.35	3.94*	2.21	2.22

Note: *) The bud reached the cupule-bract stage, as defined by Susatya (2020).

Growth rate comparison

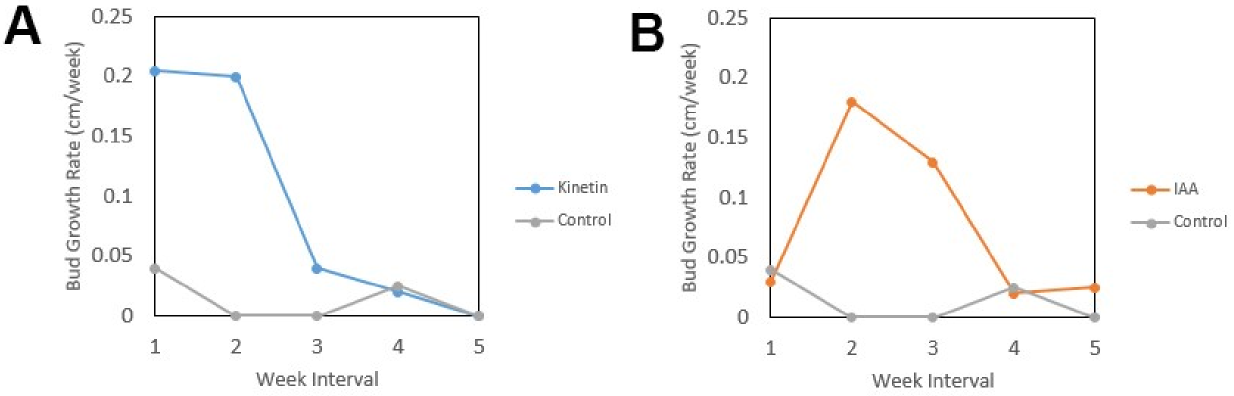

Figure 1. The growth rate comparison of (A) cytokinin (Kinetin) vs. control and (B) auxin (IAA) vs. control on *R. patma* flower buds.

The kinetin-injected buds showed the highest growth rate from the first-week to the second-week interval, despite a slight slow-down in the second-week interval, and drastic slow-down afterward (Fig. 1A). On the other hand, IAA-injected buds showed a burst of growth from the first-week to the second-week interval before slowing down to stagnation towards the fourth-week interval (Fig. 1B). Of the two IAA-treated buds, one showed rapid growth (Table 1, K3) and the other a transition from cupule stage to the cupule-bract stage (Table 1. K4; Fig. 2), despite both having the same growth age of approximately 5–6 months old (Mursidawati & Wicaksono 2020). Comparatively, the control buds showed only slight growth in the first and fourth weeks with growth stagnation between the second and third weeks.

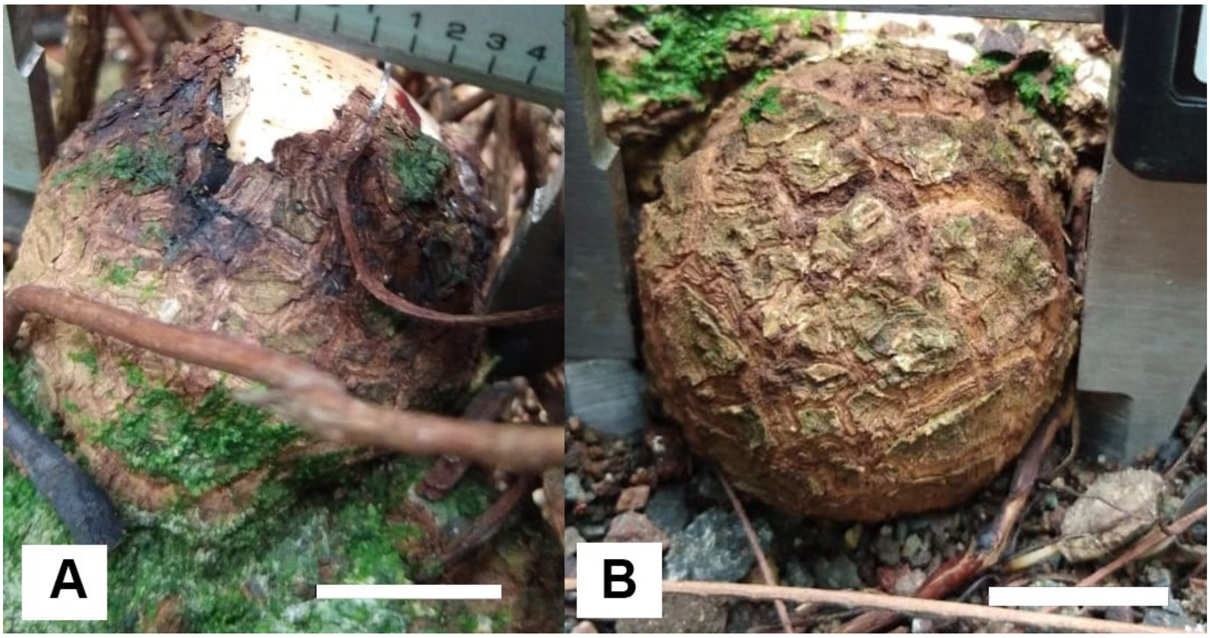

Figure 2. The one bud treated with IAA (K4) with its distal part cracked open on the second week, revealing the young pale bract indicating transition towards the cupule-bract stage **(A)**, compared to the one bud with in the control (K6), which is still in the cupule stage **(B)**. Susatya (2020) growth stages and descriptions were used. Scale bar = 1 cm.

Auxin and cytokinin functionality on the bud growth

In plant growth, auxin works by altering the cell wall rigidity, allowing water transport into the cell and resulting in the elongation of the cell (Majda & Robert 2018). Auxin plays a significant role in apical growth (upwards and downwards) and dominance (Taiz *et al*. 2015). In comparison, cytokinin regulates the cell cycle especially the interphase-mitosis transition in plant tissue (Schaller *et al*. 2014). In the present study, it appeared that the effect of cytokinin (kinetin) was almost immediate as shown by high initial growth in the first-week interval (week 0 towards 1; Fig. 1A), while auxin (IAA) delayed growth until the second-week interval (week 1 towards 2; Fig. 1B).

In the *R. patma,* flower buds treated with kinetin (cytokinin), it appeared that the cytokinin directly affected cellular division upon injection, which led to the enlargement of the flower buds. Comparatively, in the IAA (auxin)-treated flower buds, the auxin induced the distal meristem of the flower buds, resulting in the bract emergence. For now, it is not understood if the flower bud distal meristem that differentiates into bracts, perigone lobes, and the central disc organ (Mursidawati & Wicaksono 2020) is analogous in function to the plant apical meristem that differentiates into a shoot or root. Auxin has been known to be produced in young developing leaves in the plant apex and transported uni-directionally towards the root tip via the phloem, but later auxin can be synthesized in different plant tissues (Chandler 2009), while most of the cytokinin is synthesized in roots, cambium and actively dividing cells, and transported by xylem (Chen *et al*. 1985; Campbell *et al*. 2008). Hence, it is possible that some primordial tissue in *Rafflesia* plays a role in auxin production. In *Rafflesia,* auxin and cytokinin actively play a role during flower development, with cytokinin mainly regulating flower development by activating MADS-box transcriptional factor genes (Amini *et al*. 2019).

Limitations and future challenges

The problem of performing experiments with *Rafflesia* is the limited availability of plant material in the wild or the botanical garden. Of all available flower buds within a specific area, we can only select a few of them in order to keep the population sustainable. The only solution is to repeat the study in a controlled area (e.g. botanical garden or specific area in a national park with permission), where flower buds may be more readily available within the population to sample. Alternatively, a study could be repeated in separated time frames but in the same period, e.g. one in July 2020, later in July 2021, etc. Careful selection for the sample is also highly encouraged, especially due to *Rafflesia* population fragility.

The other problem to be considered is the PGR injection to the flower buds. As it causes an open wound to the flower bud, which may render the *Rafflesia* bud vulnerable due to exposure to microorganisms or herbivores. Environmental factors, like temperature and soil moisture, might also play roles in flower growth (Major 1980). In the wild, sometimes *Rafflesia* buds never reached the anthesis period and died during the flower bud development period, thus indicating that the flower bud may be sensitive to slight disturbances (Mursidawati

2014). If a wound in the bud is exposed by injection, it may further lead to death.

Lastly, we have also considered the PGR effectiveness periods. According to Mariani *et al*. (2011), single injection of cytokinin resulted in a plant response within two weeks. Other studies have revealed that in the cortex and stele of root tissue, auxin lasts for 0.12 and 21 hours, respectively. In a condition where auxin was provided using agar and it is transported in the order of hundreds to thousands of pictograms per hour (Nonhebel *et al*. 1985; Kramer & Ackelsberg 2015). As for cytokinin, transportation mode is unknown. It is unclear, however, under *in vivo* conditions with injection treatments as in Mariani *et al*. (2011) and the present study, the speed and size would be for the metabolic rate and transport of auxin and cytokinin by the *Rafflesia* exposed tissues. Giving multiple doses to *Rafflesia* flower bud could open more wounds that damage the bud, hence a better way to administer the PGR doses needs to be considered. Additionally, other than the requirement to test on a larger sample number, this study should probably be tested on other *Rafflesia* species to see if they provide similar results to *R. patma*. Also, other PGRs like gibberellic acid (GA) and jasmonic acid (JA) and the combinations between them should be tested to see if different results in flower bud growth might occur. From that, a histological analysis could be done to determine if the given PGRs or their combinations might alter tissue growth compared to the control.

CONCLUSION

Auxin and cytokinin might affect the *R. patma* flower bud growth. Injecting both IAA and kinetin, which are auxin and cytokinin, respectively, caused flower bud enlargement. However, only IAA treatment showed flower bud transition from the cupule stage to the cupule-bract stage. This preliminary overview might reveal the significance of auxin in flower bud development in *Rafflesia* compared to cytokinin. Nevertheless, more samples are needed to confirm this claim, with PGR administration needing to be made more effective to reduce stress or damage in the treated flower buds. Additionally, other PGRs and their combinations will be required in future studies, combined with histological analysis of the treated flower bud tissues.

ACKNOWLEDGEMENTS

The authors thank Dr. Siti Nur Hidayati and Dr. Jeffrey Walck (Department of Biology, Middle Tennessee State University, USA) for the corrections and editing on the final version of this manuscript.

REFERENCES

Alabadí D, Blázquez MA, Carbonell J, Ferrándiz C, Pérez-Amador MA. 2009. Instructive roles for hormones in plant development. International Journal of Developmental Biology 53: 1597–1608. doi:10.1387/ijdb.072423da.

Amini S, Rosli K, Abu-Bakar MF, Alias H, Mat-Isa MN, Juhari MAA, Haji-Adam J, Goh HH, Wan KL. 2019. Transcriptome landscape of *Rafflesia cantleyi* floral buds reveals insights into the roles of transcription factors and phytohormones in flower development. PloS One 14: e0226338. doi:10.1371/journal.pone.0226338

Bartrina I, Otto E, Strnad M, Werner T, Schmulling T. 2011. Cytokinin regulates the activity of reproductive meristems, flower organ size, ovule formation, and thus seed yield in *Arabidopsis thaliana*. Plant Cell 23: 69–80. doi:10.1105/tpc.110.079079

Campbell NA, Reece JB, Urry LA, Cain ML, Wasserman SA, Minorsky PV, Jackson RB. 2008. Biology 8th ed. pp. 827-830. Benjamin Cummings, San Francisco.

Chandler JW. 2009. Local auxin production: a small contribution to a big field. Bioessays 31: 60–70. doi:10.1002/bies.080146

Chen CM, Ertl JR, Leisner SM, Chang CC. 1985. Localization of cytokinin biosynthetic sites in pea plants and carrot roots. Plant Physiology 78: 510–513. doi:10.1104/pp.78.3.510

Fan Z, Li J, Li X, Wu B, Wang J, Liu Z, Yin H. 2015. Genome-wide transcriptome profiling provides insights into floral bud development of summer-flowering *Camellia azalea*. Scientific Reports 5: 9729. doi:10.1038/srep09729

Gambetta GA, Fei J, Rost TL, Knipfer T, Matthews MA, Shackel KA, Walker MA, McElrone AJ. 2013. Water uptake along the length of grapevine fine roots: developmental anatomy, tissue-specific aquaporin expression, and pathways of water transport. Plant Physiology 163: 1254–1265. doi:10.1104/pp.113.221283

Grossmann K. 2007. Auxin herbicide action: lifting the veil step by step. Plant Signaling & Behavior 2: 421–423. doi: 10.4161/psb.2.5.4417

Kramer EM, Ackelsberg EM. 2015. Auxin metabolism rates and implications for plant development. Frontiers in Plant Science 6: 150. doi:10.3389/fpls.2015.00150

Major DJ. 1980. Environmental effects on flowering. In: Fehr WR, Hadley HH (eds). Hybridization of Crop Plants. American Society of Agronomy and Crop Science Society of America, Publishers Madison, Wisconsin. doi:10.2135/1980.hybridizationofcrops.c1

Molina J, McLaughlin W, Wallick K, Pedales R, Marius VM, Tandang DN, Damatac A, Stuhr N, Pell SK, Lim TM, Novy A. 2017. Ex situ propagation of Philippine *Rafflesia* in the United States: Challenges and prospects. Sibbaldia 15: 77–96. doi:10.23823/Sibbaldia/2017.224

Mariani TS, Fitriani A, Teixeira da Silva JA, Wicaksono A, Chia TF. 2011. Micropropagation of *Aglaonema* using axillary shoot explants. International Journal of Basic and Applied Sciences 11: 46–53.

Majda M, Robert S. 2018. The role of auxin in cell wall expansion. International Journal of Molecular Sciences 19: 951. doi:10.3390/ijms19040951

Mursidawati S. 2014. *Rafflesia patma* (Rafflesiaceae): notes on its field study, cultivation, seed germination and anatomy. Buletin Kebun Raya 17: 9–14.

Mursidawati S, Handini E. 2009. Biologi konservasi tumbuhan holoparasit: percobaan kultur in vitro. Prosiding Konservasi Flora Indonesia Dalam Mengatasi Dampak Pemanasan Global. Kebun Raya 'Eka Karya" Bali – LIPI, Tabanan, Bali. pp. 158–162.

Mursidawati S, Wicaksono A. 2020. Tissue differentiation of the early and the late flower buds of *Rafflesia patma* Blume. Journal of Plant Development 27: 19–32. doi:10.33628/jpd.2020.27.1.19.

Mursidawati S, Ngatari N, Irawati I, Cardinal S, Kusumawati R. 2015. *Ex situ* conservation of *Rafflesia patma* Blume (Rafflesiaceae): an endangered emblematic parasitic species from Indonesia. Sibbaldia 13: 99–110. doi:10.23823/Sibbaldia/2015.77

Mursidawati S, Wicaksono A, Teixeira da Silva JA. 2019. Development of the endophyte parasite, *Rafflesia patma* Blume, among host plant (*Tetrastigma leucostaphylum* (Dennst.) Alston) vascular cambium tissue. South African Journal of Botany 123: 382–386. doi:10.1016/j.sajb.2019.03.028

Mursidawati S, Wicaksono A, Teixeira da Silva JA. 2020. *Rafflesia patma* Blume flower organs: histology of the epidermis and vascular structures, and a search for stomata. Planta 25: 112. doi:10.1007/s00425-020-03402-5

Nikolov LA, Tomlinson PB, Manickam S, Endress PK, Kramer EM, Davis CC. 2014. Holoparasitic Rafflesiaceae possess the most reduced endophytes and yet give rise to the world's largest flowers. Annals of Botany 114: 233–242. doi:10.1093/aob/mcu114

Nonhebel HM, Hillman JR, Crozier A, Wilkins MB. 1985. Metabolism of [C-14] indole-3-acetic acid by the cortical and stelar tissues of *Zea mays* L. roots. Planta 164: 105–108. doi:10.1007/BF00391033

Novák J, Pavlů J, Novák O, Nožková-Hlaváčková V, Špundová M, Hlavinka J, Koukalová Š, Skalák J, Černý M, Brzobohatý B. 2013. High cytokinin levels induce a hypersensitive-like response in tobacco. Annals of botany 112: 41–55. doi: 10.1093/aob/mct092

Schaller GE, Street IH, Kieber JJ. 2014. Cytokinin and the cell cycle. Current Opinion in Plant Biology 21: 7–15. doi:10.1016/j.pbi.2014.05.015

Sukamto LA. 2001. Upaya menumbuhkan *Rafflesia arnoldii* secara in vitro. Prosiding Nasional Puspa Langka. Bogor. June 16 2001, pp 31–34

Sukamto LA, Mujiono M. 2010. *In vitro* culture of holoparasite *Rafflesia arnoldii* R. Brown. Buletin Kebun Raya 13: 79–85.

Susatya A. 2020. The growth of flower bud, life history, and population structure of *Rafflesia arnoldii* (Rafflesiaceae) in Bengkulu, Sumatra, Indonesia. Biodiversitas 21: 792–798. doi:10.13057/biodiv/d210247

Taiz L, Zeiger E, Møller IM, Murphy A. 2015. Plant physiology and development. Sinauer Associates, Sunderland.

van der Krieken WM, Croes AF, Smulders MJM, Wullems GI. 1989. Cytokinins and flower bud formation *in vitro* in tobacco. Plant Physiology 92: 565–569.

Wicaksono A, Teixeira da Silva JA. 2015. Attempted callus induction of holoparasite *Rafflesia patma* Blume using primordial flower bud tissue. Nusantara Bioscience 7: 96–101. doi:10.13057/nusbiosci/n070206

Wicaksono A, Teixeira da Silva JA, Mursidawati S. 2017. Dispersal of *Rafflesia patma* Blume endophyte in grafted host plant (*Tetrastigma leucostaphylum* (Dennst.) Alston). Journal of Plant Development 24: 19–32. doi:10.33628/jpd.2020.27.1.19

Wicaksono A, Mursidawati S. 2020. Lugol's iodine test on *Rafflesia patma–Tetrastigma leucostaphylum* intersection tissue for preliminary starch visualization. Nusantara Bioscience 12: 91–96. doi:10.13057/nusbiosci/n120202

Wicaksono A, Mursidawati S, Molina JA. 2020. Plant within a plant: Insights on the development of the *Rafflesia* endophyte within its host. The Botanical Review. doi:10.1007/s12229-020-09236-w