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Article

Rapid propagation of *Homalomena gigantea* ornamental plant using rhizome cuttings

Propagação rápida da planta ornamental *Homalomena gigantea* usando estacas de rizoma

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Abstract: This study investigates the critical factors influencing shoot and root regeneration from *Homalomena gigantea* rhizome cuttings, providing valuable insights for rhizome cutting practices for this plant species. Three key experiments were conducted to assess the impact of plant growth regulator (PGR) concentrations, cutting length, and cutting section on regeneration. In the first experiment, different concentrations of α-NAA and BAP were examined, revealing that PGR selection and concentration play a pivotal role in shoot and root induction and plantlet development. The highest success rates were observed at 150 ppm α-NAA and 100 ppm BAP, although there was a trade-off between the quantity and quality of plantlets, emphasizing the need for a balanced plant regulator selection. The second experiment investigated the influence of rhizome cutting length. Longer cuttings, particularly at 5 and 7 cm, consistently led to better shoot and root development, highlighting the importance of selecting appropriate cutting lengths. In the third experiment, cutting sections (apical, middle, and distal) without PGR concentrations were assessed, with all sections showing high regeneration rates. However, the apical section produced the highest-quality plantlets with superior growth parameters, followed by the middle section, while the distal section exhibited lower effectiveness. In summary, these findings underscore the significance of PGR choice and concentration, cutting length, and cutting section for successful regeneration of *H. gigantea* from rhizome cuttings. These insights provide valuable guidance for enhancing rhizome cuttings methods for this plant species and promoting efficient propagation.
Keywords: apical rhizome, distal rhizome, *Homalomena gigantea*, middle rhizome.

Resumo: Este estudo investiga os fatores críticos que influenciam a regeneração de brotos e raízes a partir de cortes de rizoma de *H. gigantea*, fornecendo *insights* valiosos para as práticas de corte de rizoma. Três experimentos foram conduzidos para avaliar o impacto das concentrações de reguladores vegetais, comprimento do corte e seção de corte na regeneração. No primeiro experimento, diferentes concentrações de α-NAA e BAP foram examinadas, revelando que a seleção e concentração de reguladores vegetais desempenham um papel crucial na indução de brotos e raízes e no desenvolvimento de plântulas. As taxas de sucesso mais altas foram observadas a partir de 150 ppm de α-NAA e 100 ppm de BAP, embora houvesse um equilíbrio entre a quantidade e a qualidade das plântulas, enfatizando a necessidade de uma seleção equilibrada de reguladores vegetais. O segundo experimento investigou a influência do comprimento de corte do rizoma. Cortes mais longos, especialmente com cinco e sete cm, levaram a um melhor desenvolvimento de brotos e raízes, destacando a importância da seleção de comprimentos de corte apropriados. No terceiro experimento, seções de corte (apical, média e distal) sem concentrações de reguladores vegetais foram avaliadas, com todas as seções mostrando altas taxas de regeneração. No entanto, a seção apical produziu plântulas de melhor qualidade com parâmetros de crescimento superiores, seguida pela seção média, enquanto a seção distal apresentou menor eficácia. Em resumo, essas descobertas destacam a importância da escolha e concentração de reguladores vegetais, comprimento de corte e seção de corte para a regeneração bem-sucedida de *H. gigantea* a partir de cortes de rizoma. Esses *insights* fornecem orientações valiosas para aprimorar os métodos de corte de rizoma para essa espécie de planta e promover uma propagação eficiente.
Palavras-chave: *Homalomena gigantea*, rizoma apical, rizoma distal, rizoma médio.

Introduction

Homalomena is a large and diverse genus of tropical plants within the *Araceae* family, encompassing over 150 species, primarily thriving in the humid environments of Southeast Asia (Boyce and Yeng, 2008). Vietnam, one of the regions rich in *Araceae* biodiversity, is the native habitat for numerous species of this botanical family with a wide variety of plants cultivated for food, traditional medicine and ornamental purposes. Given this context, species within the *Homalomena* genus hold significant potential for various applications (Van, 2022).

H. gigantea, one of 130 species of aroids in the Bogor Botanic Gardens, has long been utilized as both ornamental plants and a source of essential oils (Yuzammi, 2018). Additionally, one of the 17 species of *Araceae* plants, is cultivated for ornamental purposes among the Indonesian people (Rambey et al., 2022). Nguyen et al. (2019) reported that *H. gigantea* is one of the 94 ornamental plant species present in the Pu Hoat Nature Reserve, Nghe An Province, Vietnam.

Previous studies have demonstrated that *H. gigantea* possesses potential for human lung cancer cell line (Nguyen et al., 2022), as well as anti-inflammatory (Nguyen et al., 2023) and anti-osteoporotic activities (Pham et al., 2022; Nguyen et al., 2023). Due to the overexploitation of wild *H. gigantea* resources in Vietnam, driven by the extensive therapeutic value of the species, it is now listed as a precious and vulnerable medicinal plant (Nguyen et al., 2019).

Aglaonema, Alocasia, Anthurium, Dieffenbachia, Homalomena, Philodendron, Spathiphyllum, and *Syngonium* are among the Araceae genera for which micropropagation has been done (Chen and Wei, 2018; Kaviani et al., 2019). *Zamioculcas zamiifolia* Engl., a drought-resistant plant in the family Araceae, was undergoing development of a comprehensive *in vitro* propagation protocol (Sayadi Nejad, and Sadeghi, 2019; Ghoochani Khorasani et al., 2021; Pourhassan et al., 2023; Kharrazi et al., 2023). To stimulate the development of numerous shoots for micropropagation, rhizome bud explants of *Homalomena aromatica* Schott were cultured on Murashige and Skoog (MS) media supplemented with varying concentrations of 6-benzylaminopurine. Among the different combinations, the highest success in shoot regeneration was achieved on MS medium supplemented with 2.0 mg L^{-1} 6-benzylaminopurine (BAP) with subsequent rooting on half-strength MS medium supplemented with 0.5 mg L^{-1} α-naphthalene acetic acid (NAA), as reported by Raomai et al. (2013). Stanly et al. (2012) suggested that the ideal conditions for *Homalomena pineodora* Sulaiman & Boyce propagation using rhizome

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bud explants involve MS medium supplemented with 3% sucrose and 0.5 mg L^{-1} BAP without the need to add auxin. In order to quickly produce large quantities of enhanced materials and to maintain the traits of the parent plant, vegetative multiplication through stem cutting is essential (Hartmann et al., 2002). *Sanguinaria Canadensis* L., *Trillium grandiflorum* (Michx.) Salisb, *Asarum canadense* L., *Caulophyllum thalictroides* L. (Nivot et al., 2008), and *Trillium govanianum* Wall. ex D. Don (Chauhan et al., 2020) have all been successfully vegetatively propagated by rhizome cuttings. However, there has been no study on propagating the species *H. gigantea* through rhizome cuttings.

In this study, we examined the influence of α-NAA and BAP, rhizome length, and different rhizome cutting section (apical, middle, distal rhizome) from mature plants on the regeneration of *H. gigantea*. Consequently, we have developed a successful regeneration strategy for the rapid propagation of this species.

Materials and Methods

Plant Material and Explant Source

The rhizomes of *H. gigantea* used in this study were sourced from a forest located in the A Roang commune, A Luoi district, Thua Thien Hue province (Fig. 1).

Fig. 1. The morphology of *Homalomena gigantea:* (A) Leaf; (B) Rhizome; (C-D) Flowers.

Explant Surface Sterilization

H. gigantea rhizomes were collected and initially washed for two to five minutes under running water from the faucet.

Response to α-NAA and BAP concentration

To assess the stimulatory effects of different concentrations of α-NAA and BAP (Sigma-Aldrich) applied individually, we used *H. gigantea* rhizome cuttings. These cuttings, each measuring 3 cm in length, were taken from mature plants. Employing a randomized complete-block design, a total of five treatments were immersed in experimental concentrations for 24 hours: a control group (no α-NAA/BAP administered), 50, 100, 150, and 200 ppm for α-NAA and BAP separately. Each treatment comprised twenty cuttings, all obtained from the same section of the rhizome but from different plants. Data were collected from three replicates after 16 weeks of rhizome cutting, with each replicate containing 30 explants.

Response to different length of rhizome cutting

The samples were collected from mature plants, segmented at identical positions, devoid of nodes, and measured at lengths of 1, 3, 5, and 7 cm. Data were gathered from three replicates after 16 weeks of rhizome cutting, with each replicate comprising twenty explants.

Response of cuttings derived from different sections of rhizome

The rhizome was divided into three sections - apical, middle, and distal, each measuring 5 cm in length and lacking nodes, to assess shoot regrowth and root formation. Information from three experimental sets was documented after 16 weeks of rhizome cutting, with each set comprising twenty explants.

Experiment design and statistical data analysis

Each experiment was conducted in a completely randomized design and repeated three times, with 30 explants in each condition. The data were subjected to statistical analysis using one-way analysis of variance (ANOVA) in IBM SPSS version 19.0, and Duncan's multiple range tests were employed to identify significant differences among the means.

Results and Discussion

Effect of different concentrations of α-NAA and BAP on shoot and root regeneration from *H. gigantea* rhizome cuttings

Tab. 1 presents the results of an experiment that investigated the effect of different concentrations of the PGR α-NAA and BAP on shoot and rooting regeneration from the rhizome cutting of *H. gigantea*. The table provides data on various parameters, including the percentage of explant response, shoot and root number and length (Tab. 1, Fig. 2).

Tab. 1. Effect of different concentrations of α-NAA and BAP concentration alone on shoot and rooting regeneration from the rhizome cutting of *Homalomane gigantea.*

Plant growth regulator	Treatment [ppm]	Percent response [%]		Quality of rhizome cutting plantlets per explant (Mean ± SE)			
		Shoot	rooting	No. of shoots	Shoot length	No. of roots	Root length
α-NAA	CT	87.38 ± 0.63 c	73.94 ± 0.11 d	1.00 ± 0.97 c	2.63 ± 0.25 b	2.20 ± 0.20 e	8.63 ± 0.50 g
	50	95.80 ± 0.49 b	85.46 ± 0.04 b	1.29 ± 0.12 bc	5.40 ± 0.35 a	3.20 ± 0.26 cd	10.46 ± 0.53 ef
	100	94.32 ± 0.28 b	97.36 ± 0.87 a	1.26 ± 0.11 bc	4.60 ± 0.29 ab	2.47 ± 0.27 e	18.2 ± 0.59 a
	150	97.45 ± 0.57 a	97.14 ± 0.36 a	1.53 ± 0.13 ab	6.06 ± 0.33 a	5.53 ± 0.26 b	12.03 ± 0.60 cd
	200	85.65 ± 0.99 c	97.78 ± 0.85 a	1.20 ± 0.10 bc	4.06 ± 0.22 ab	3.40 ± 0.29 c	9.20 ± 0.18 fg
	Regression	0.054	0.891	0.532	0.528	0.571	0.1
BAP	50	89.66 ± 0.39 c	99.34 ± 0.27 a	1.27 ± 0.11 bc	4.00 ± 0.23 ab	1.93 ± 0.18 e	12.63 ± 0.49 c
	100	99.36 ± 0.10 a	98.42 ± 0.59 a	1.80 ± 0.20 a	4.23 ± 0.22 ab	2.60 ± 0.19 de	15.77 ± 0.40 b
	150	99.52 ± 0.20 a	97.49 ± 0.85 a	1.83 ± 0.15 a	5.67 ± 0.21 a	6.93 ± 0.33 a	14.53 ± 0.60 b
	200	91.93 ± 0.12 c	81.59 ± 0.41 c	1.34 ± 0.6 bc	3.23 ± 0.38 b	1.20 ± 0.11 f	11.13 ± 0.41 de
	Regression	0.536	0.183	0.541	0.349	0.443	0.386

Note: According to Duncan's multiple range tests, means that are followed by the same letter are not substantially different for each column and treatment ($P \leq 0.05$). Values reflect the means and standard errors of cultured explants, with n = 30 for each treatment.

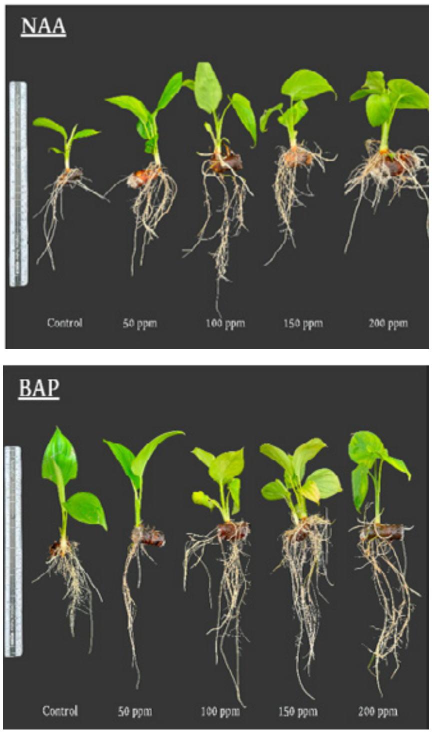

Fig. 2. Shoot and root regeneration from rhizome cuttings of *Homalomena gigantea* with different concentrations of α-NAA and BAP concentration alone.

The table 1 reveals that the responses to tested PGR and their concentrations varied significantly. It is evident that shoot rooting exhibits a strong positive correlation (r = 0.891) with the PGR treatments, indicating that higher concentrations of the regulator tend to result in increased shoot rooting percentages. Similarly, the number of shoots and shoot length show moderate positive correlations (r = 0.532 and r = 0.528, respectively) with the treatments, suggesting that higher concentrations may lead to greater numbers of shoots and longer shoot lengths. Moreover, the number of roots demonstrates a moderate positive correlation (r = 0.571) with the PGR treatments, implying that elevated concentrations may promote the development of more roots. However, the correlation coefficient for root length is notably lower (r = 0.1), indicating a weak positive correlation between PGR treatments and root length. The observed changes in response variables across different treatment levels further elucidate the effects of the PGR on the quality of rhizome cutting plantlets. Overall, this analysis underscores the complex relationship between regulator treatments and various aspects of plant growth, highlighting the need for careful consideration of treatment concentrations to optimize desired outcomes in rhizome cutting propagation.

The findings indicate that BAP treatments have differing impacts on various aspects of plant growth. Specifically, BAP treatments show a moderate positive correlation with shoot rooting (r = 0.536) and shoot length (r = 0.541), suggesting that higher concentrations of BAP tend to result in increased shoot rooting percentages and longer shoot lengths (Tab. 1). Conversely, the correlation coefficients for the number of shoots (r = 0.183) and number of roots (r = 0.349) with BAP treatments are comparatively lower, indicating a weaker positive correlation between BAP concentrations and these variables (Tab. 1). Despite this, there is still a discernible influence of BAP on the number of shoots and roots, albeit to a lesser extent. Interestingly, root length also demonstrates a moderate positive correlation with BAP treatments (r = 0.386), implying that higher concentrations of BAP may contribute to longer root lengths in rhizome cutting plantlets. The data provided in the table 1 offer valuable insights into the effects of varying BAP concentrations on the overall quality of rhizome cutting plantlets. It suggests that careful consideration of BAP concentrations is essential in optimizing desired outcomes in rhizome cutting propagation, as higher concentrations may promote certain aspects of plant growth while showing varying effects on others. This analysis underscores the complexity of the relationship between BAP treatments and the growth of rhizome cutting plantlets, highlighting the need for further research to fully understand and harness the potential of BAP in plant propagation practices.

For α-NAA, it is apparent that the highest concentration (150 ppm) led to the most favorable results, with the highest percentages of shoot and root development. However, the quality of rhizome cutting plantlets per explant was slightly lower in comparison to other concentrations. In contrast, for BAP, the 100 ppm concentration yielded the most promising outcomes, including the highest percentage of shoot and root development, and the best quality of rhizome cutting plantlets per explant (Tab. 1, Fig. 2). Similarly, the study mentions that the apical portion of the rhizome showed good survival efficiency with the formation of multiple roots in *T. govanianum*. The maximum root length was obtained with the treatment of NAA 250 ppm (Chauhan et al., 2020). This demonstrates the importance of PGR concentration for root development. In contrast, the result of Chauhan et al. (2020) focused on the maximum root length, where NAA at 250 ppm produced the longest roots. It is noteworthy that the middle and distal portions of the rhizomes did not respond well (Chauhan et al., 2020). Additionally, the observation of multiple roots and the formation of multiple buds from a single rhizome provide intriguing findings (Chauhan et al., 2020). The study on the influence of BAP and NAA on propagation by rhizome cutting found that different combinations of BAP and NAA had varying effects on plantlet growth. In the study by Xie et al. (2022) it was observed that rhizome weight did not significantly affect the propagation coefficient, but sectioning rhizomes into three parts resulted in a higher propagation coefficient and less retardation on growth. In the study by Dahniar and Elvavina (2022), it was determined that the optimal medium for mass plant propagation of Smooth Cayenne pineapple was BAP 3 mg L^{-1} without NAA, while the best medium for genetic engineering research was a combination of BAP 3 mg L^{-1} and NAA 2 mg L^{-1}. The research by Kentelky et al. (2021) showed that NAA increased rooting in ornamental woody shrubs, although the response varied among different species. The study by Rehman et al. (2023) found that the concentration of BAP and NAA at 1.5 mg L^{-1} and 0.5 mg L^{-1}, respectively, showed better enhancement for shoot regeneration and rooting in Kalpvriksha (*Adansonia digitata* L.) tree. Lastly, the experiment by Ashok and Ravivarman (2020) demonstrated that the rooting and survival capacity of *Duranta erecta* cuttings can be improved by using NAA at a concentration of 3000 ppm. These results collectively underscore the importance of PGR concentration in shoot and root development.

Additionally, according to Srivastava (2002), applying cytokinins (alone or in combination with auxin) to the apical end of a decapitated stem does not relieve inhibition in lateral buds and might even make it stronger. Lateral shoot development was stimulated in *Solanum tuberosum* by applying the synthetic cytokinin benzylaminopurine to the basal portion of the cuttings, as reported by Woolley and Wareing (1972). However, whether given to the lateral buds of plants that had an undamaged apex or to decapitated plants that had inhibitory doses of apical auxin applied to them, cytokinins encouraged the formation of buds (Srivastava, 2002). Therefore, it has been demonstrated that applying exogenous cytokinins to lateral buds directly or to the basal portion of the stem, as in this work, releases lateral buds from inhibition.

The influence of cutting length on shoot and root regeneration from *H. gigantea* rhizome

The experiment involved four different rhizome cutting lengths, namely 1 cm, 3 cm, 5 cm and 7 cm. Tab. 2 demonstrates that the response to these different lengths varied notably. In particular, longer rhizome cuttings appeared to yield more favorable results. The 5 cm and 7 cm cuttings consistently exhibited the highest percentages of shoot and root development, with the 5 cm length showing the greatest impact on both these parameters.

Additionally, the quality of rhizome cutting plantlets, as reflected in the length of shoots and roots, improved as the length of the cutting increased (Tab. 2, Fig. 3).

Table 2. Effect of different length rhizome cutting explant on shoot and root regeneration from the rhizome cutting of *Homalomena gigantea.*

Treatment [cm]	Percent response [%]		Quality of rhizome cutting plantlets per explant (Mean ± SE)			
	Shoot	Root	No. of shoots	Shoot length	No. of roots	Root length
1	82.42 ± 0.62 b	84.74 ± 0.23 b	0.93 ± 0.67 c	2.33 ± 0.24 b	2.00 ± 0.23 d	6.07 ± 0.38 c
3	96.83 ± 0.26 a	97.84 ± 0.14 a	1.53 ± 0.17 b	4.73 ± 0.32 a	7.73 ± 0.35 b	15.27 ± 0.64 a
5	97.87 ± 0.14 a	98.63 ± 0.11 a	2.27 ± 0.23 a	4.13 ± 0.35 a	10.40 ± 0.51 a	14.10 ± 0.43 a
7	95.84 ± 0.33 a	96.70 ± 0.25 a	1.53 ± 1.65 b	4.20 ± 0.23 a	5.20 ± 0.31 c	10.67 ± 0.60 b

Note: According to Duncan's multiple range tests, means that are followed by the same letter are not substantially different for each column and treatment ($P \leq 0.05$). Values reflect the means and standard errors of cultured explants, with n = 30 for each treatment.

Fig. 3. Shoot and root regeneration from rhizome cuttings of different length in *Homalomena gigantea.*

Similarly, Lee and Kim (2012) studied various rhizome cuttings categorized by their length and node count to enhance the propagation efficiency of the endangered *Menyanthes trifoliata* species in Korea. Among lengthy cuttings spanning 10-18 cm, the rhizome exhibited the greatest growth length and volume. However, in comparison to longer cuttings, shorter ones (2-4 cm) and medium-length ones (4-10 cm) displayed a higher proportional growth length of rhizomes. The most significant relative increase in rhizome volume was observed in the medium-length cuttings. The efficiency of rhizome growth relative to the number of nodes remained unaffected. As per this study, utilizing medium-length cuttings stands as the most effective method in promoting *M. trifoliata* growth (Lee and Kim, 2012). In the study on *Cyperus aromaticus*, it was found that the cumulative shoot emergence and the number of underground tillers produced were positively correlated with the initial length of the rhizome fragments (Chadha et al., 2022). In the study on pepper-rosmarin, the cuttings with a length of 14.1 to 17 cm showed the highest percentage of rooting and the largest average of other variables (Carvalho et al., 2009). Finally, in the study on alstroemeria, the length of rhizome of 2.1 to 2.6 cm with 3-4 feeder roots resulted in the highest percentage of successful survival and growth parameters (Singh et al., 2007).

The impact of rhizome cutting section on shoot and root regeneration in *H. gigantea*

The data in Table 3 reveals that all three rhizome cutting sections, apical, middle, and distal, exhibited remarkably high percentages of response, with values close to 100%. This suggests that all cutting locations are conducive to regeneration rhizome in *H. gigantea*. However, there are notable differences in the quality of the plantlets produced (Tab. 3, Fig. 4).

The apical rhizome produced the most impressive results, with the highest mean values for the number of roots, and shoot and root length. This indicates that the apical rhizome cutting is the most favorable for promoting robust shoot and root development (Tab. 3, Fig. 4).

The middle rhizome cutting also performed well, with intermediate values for these parameters, while the distal rhizome cutting showed lower values, indicating that it may be less effective for shoot and root regeneration compared to the other locations (Tab. 3, Fig.4).

Tab. 3. Effect of different rhizome cutting' location explant on shoot and rooting regeneration from the rhizome cutting of *H. gigantea.*

Section of rhizome	Percent response [%]		Quality of rhizome cutting plantlets per explant (Mean ± SE)			
	Shoot	Root	No. of shoot	Shoot length	No. of root	Root length
Apical	99.82 ± 0.04 a	99.66 ± 0.05 a	1.40 ± 0.13 b	6.70 ± 0.25 a	34.67 ± 0.72 a	19.86 ± 0.94 a
Middle	99.78 ± 0.06 a	99.70 ± 0.10 a	1.87 ± 0.19 a	3.67 ± 0.25 b	10.33 ± 0.48 b	14.13 ± 0.32 b
Distal	99.74 ± 0.07 a	99.76 ± 0.10 a	1.60 ± 0.13 ab	3.06 ± 0.22 b	4.67 ± 0.27 c	10.60 ± 0.43 c

Note: According to Duncan's multiple range tests, means that are followed by the same letter are not substantially different for each column and treatment ($p \leq 0.05$). Values reflect the means and standard errors of cultured explants, with n = 30 for each treatment.

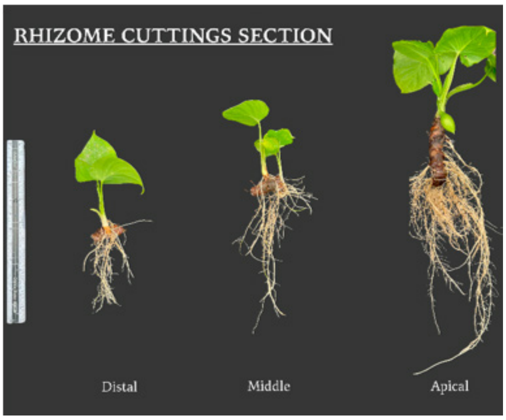

Fig. 4. Shoot and root regeneration from cuttings of different sections of *Homalomena gigantea* rhizome.

The apical rhizome is the most promising location for producing high-quality plantlets, while the middle rhizome also provides favorable results, and the distal rhizome is less efficient for this purpose (Tab. 3, Fig. 4). On the contrary in the case of *T. govanianum*, it was found that only the apical portion of the rhizome was capable of forming roots and apical buds. The middle and distal portions did not respond effectively (Chauhan et al., 2020). These findings are essential for rhizomes cuttings propagation of *H. gigantea* practices.

Conclusions

The experiment suggests that the optimal concentration of α-NAA for *H. gigantea* rhizome cutting propagation was 150 ppm, while for BAP, it was 100 ppm. These concentrations resulted in the highest percentages of shoot and root development, along with the best quality of rhizome cutting plantlets per explant.

Longer rhizome cuttings tend to yield more favorable results in terms of shoot and root development. Additionally, the apical portion of the rhizome was found to be the most conducive for producing high-quality plantlets, followed by the middle portion, while the distal portion is less efficient for regeneration.

Author Contribution

TP: conceptualization, methodology, investigation, writing—original draft, revision. **NTTL:** collecting samples. **TND, HNMN, HYTN, TTAB, TYNY:** methodology, investigation. **TQTN:** conceptualization, writing-review, revision and editing, project administration.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data Availability Statement

Data will be made available on request.

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