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Acclimatization of Tiger Orchid (*Grammatophyllum speciosum*. Blume) Plantlets on Various Growing Media and Shade Levels

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Abstract:
Background*: Tiger orchid (Grammatophyllum speciosum. Blume) is designated as a protected species based on Government Regulation of the Republic of Indonesia, number 7 of 1999. Efforts to preserve the tiger orchid can be carried out by plant propagation using tissue culture techniques. This study aims to analyze the effects of growing media and shade levels on the growth and vigor of acclimatized plantlets. The research was conducted at the Permanent Nursery Greenhouse of BPDAS Barito, Guntung Manggis, Banjarbaru City, South Kalimantan.*
Materials and Methods: *The experimental method is employed as the research method with an experimental design using a split plot design, the main plot is the shade levels consisting of 3 levels (paranet 50%, paranet 70%, and paranet 90%), and the growing media consisting of 4 levels (peat moss, fern, husk charcoal, and cocopeat) with 3 replications. The observed parameters included plant height, leaf length, leaf growth, survival percentage, and plantlet vigor.*
Results*: The results showed that the interaction of growing media and shade level treatments had significant effects on plantlet growth, except for the parameters of number of leaves (plant height p < 0.01, leaf length p 0.04, leaf growth p 1.344, and survival percentage p 0.015). The main plot treatment had significant effects on the parameters (height p 0.002, leaf length p 0.035, number of leaves p 0.014, and survival percentage p 0.011). Sub-plot treatments had significant effects on plant height p < 0.01, leaf length p < 0.01, and survival percentage p 0.001, but had no significant effect on the parameter of number of leaves p 0.517.*
Conclusion: *The best treatment combination was paranet 50%+ cocopeat. The vigor of plantlets was classified into some categories, namely very low (3 plantlets), low (7 plantlets), medium (13 plantlets), good (12 plantlets), and very good (1 plantlet).*
Key Word*: acclimatization; Grammatophyllum speciosum; plantlets; shade levels; growing media.*

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I. Introduction

Indonesia is one of the countries that has the largest tropical rain forest out of five countries in the world, namely Brazil, Congo, Indonesia, Peru, and Colombia, and has orchid species richness that is very diverse. Orchid plant is the member of the Orchidaceae family. This family consists of 800 genera, some of which are almost extinct, and one of the endangered orchids is the Grammatophyllum genus.

Orchids in the forest are experiencing population decline due to over-exploitation, habitat loss and climate change [6]. This is in line with research [1] stating that the decline in the orchid population is the results of overexploitation due to economic value, limited habitat, small population size, symbiont needs, complex reproductive mechanisms, and specific habitat requirements. The propagation of the Grammatophyllum sp orchid by tissue culture techniques has been successfully carried out by several studies [8,9,6].

Acclimatization of in vitro culture is an adaptation process of in vitro cultured plants (plantlets) to new environmental stresses before being planted in the field. The new environmental conditions include temperature, light and humidity. This acclimatization stage is also a crucial stage in tissue culture. Plantlet death after acclimatization often occurs, so this stage needs to be performed carefully [2]. Factors that influence the success of plantlet growth in the acclimatization phase are plantlet condition, light intensity, relative humidity, type of growing media, nutrition and fertilization as well as control of pests and microorganisms [3].

Different light intensity/shade level is required for each type of orchid and can affect the growth of orchid plants [18,11,12,10,4].

According to Satsijati [6] media are one of the environmental factors that serves to provide nutrients and water for plant growth. A mixture of two types of media can improve the shortcomings of each of these media,

among others, in the ability to maintain the humidity of the media. Media that can be used for acclimatization of orchid species are fern, peat moss, kadaka root, cocopeat, charcoal, and tile fragments [15,14,13,17,16]. The effects of the growing media characteristics at different shade levels on the growth and vigor of tiger plantlets has not been widely studied. This study aims to analyze the effects of growing media, namely peat moss, fern, husk charcoal, and cocopeat under different paranets, namely paranet 50%, paranet 70%, and paranet 90% on the growth and vigor of Grammatophyllum speciosum. Blume plantlets during the acclimatization phase.

II. Material And Methods

The research was carried out in the Permanent Nursery Greenhouse, Forestry Research and Development, Guntung Manggis, Banjarbaru.

Study Design: The method used in the study was the experimental method with a split plot design with two factors.

Study Location: The research was carried out in the Permanent Nursery Greenhouse, Forestry Research and Development, Guntung Manggis, Banjarbaru.

Study Duration: June to September 2021 (for 12 weeks after planting).

Sample size: The research material was tiger orchid plantlets from the Tissue Culture Laboratory, Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru. The growing media consisted of peat moss, fern, husk charcoal, and cocopeat. Paranet 50%, paranet 70%, and paranet 90 % were used for different light intensities. The other equipment included luxmeter (extech light meter LT-300), room thermometer and soil tester, scales, ruler, stationery, camera.

Sample size calculation: The first factor as the main plot was the shade level consisting of 3 levels: n_1 = paranet 50%; n_2 = paranet 70%; and n_3 = paranet 90%, the second factor as a sub-plot was the growing media consisting of 4 levels: m_1 = peat moss; m_2 = fern; m_3 = husk charcoal; and m_4 = cocopeat.

Subjects & selection method: The treatment combinations in this study were 12 combinations and each treatment combination was repeated 3 times. Each experimental plot consisted of 10 plantlets, so there were 360 plantlets of tiger orchids. The research parameters included growth parameters (plantlet height, leaf length, number of leaves, and survival percentage) and plantlet vigor parameters.

Procedure methodology

The measurement of parameters of plantlet height, leaf length and percentage of survival were carried out every 2 weeks (2 WAP, 4 MST, 6 MST, 8 MST, 10 MST, and 12 MST).The measurement of the number of leaves was carried out every 4 weeks (4 WAP, 8 WAP and 12 WAP). Testing of the plantlet vigor parameters using a score of epidermal thickness, cuticle thickness, and leaf color.

Statistical analysis

Data were analyzed using Analysis of variance of various effects of growing media at shade levels on plantlet height, leaf length, number of leaves, and survival percentage. Duncan's multiple range test was performed to find out the effects of treatments of different growing media at shade levels on plantlet height, leaf length, number of leaves, and survival percentage after acclimatization for 12 WAP. Since the standard for the vigor assessment in the acclimatization phase of tiger orchids is seemingly not available yet, but there is a Guidebook for Rice Plant Characterization and Evaluation System (DEPARTMENT OF AGRICULTURE, 2003). Departing from this guide, for tiger orchids, the authors describes vigor as the ability of tiger orchids to avoid light and drought stress with indicators of epidermal thickness, cuticle thickness, and leaf color.

III. Result and Discussion

Observations of the Acclimatization Environment

The average daily temperature in the greenhouse ranged from 25.14°C to 29.21°C and the average daily humidity ranged from 71.62% to 80.86%, indicating that the climate (temperature and humidity) of the acclimatization environment of tiger orchids was in a fairly ideal condition. The climate range (climate space) of the endemic moon orchid (Phalaenopsis amabilis (L.) blume grown in a greenhouse consists of temperature ranging from 26°C to 29°C and relative humidity ranging from 60% to 90% [19].

The light intensity outside the greenhouse was 93,200 lux ≈ 1,725 µmol m^{-2} s^{-1} (Photosynthetic photon flux densities/PPFD), and that inside the greenhouse was 27,960 lux 517 µmol m^{-2} s^{-1}. Only ± 30% of light entering into the greenhouse room was filtered by the building roof that was not made of glass (glass transmission). The light under paranet 50% was 13,980 lux ≈ PPFD 259 µmol m^{-2} s^{-1}, on paranet 70% was 8,388 lux ≈ PPFD 155 µmol m^{-2} s^{-1}, and the light under paranet 90% was 2,796 lux ≈ PPFD 52 µmol m^{-2} s^{-1}. The convertion of lux unit to PPFD was carried out using the PPFD conversion tool (Mecha Tronix Horticulture Lighting, 2021).

Major Observations

The results of the analysis of variance showed that there were effects of the interaction between shade levels and growing media on the growth parameters, except for the number of leaves (*p* 1.344). The shade levels independently had significant effects on all parameters, and had a very significant effect on plantlet height (*p* 19.360). The growing media independently had very significant effects on all parameters, except on the number of leaves which had no significant effect (*p* 517). The statistical data can be seen in (Table 1). According to Irsyadi [3] the factors that influence the success of plantlet growth in the acclimatization phase are plantlet condition, light intensity, relative humidity, type of growing media, nutrition and fertilization, and control of pests and microorganisms.

Table no 1: Analysis of variance of various effects of growing media at shade levels on plantlet height, leaf length, number of leaves, and survival percentage

Diversity Sources	Plantlet height (cm)		Leaf length (cm)		Number of leaves (strands)		Survival percentage (%)	
	F_hit	*P*	F_hit	*P*	F_hit	*P*	F_hit	*P*
Main Plot								
Shade (N)	19.360 **	0.002	6.131 *	0.035	9.405 *	0.014	10.500 *	0.011
Sub-plots								
Media (M)	173.021 **	< 0.01	43.034 **	< 0.01	0.787 ns	0.517	9.180 **	0.001
Interaction (MxN)	10.299 **	< 0.01	4.880 **	0.004	1.344 ns	0.289	3.660 *	0.015

Level of Significance 5%

Increase in Plantlet Height

The results of the Duncan's Multiple Range Test /DMRT at the 5% level showed the difference among treatments presented in (Table 2). The effects of paranet 50% on growing media of peat moss, fern, husk charcoal, and cocopeat were significantly different. The effects of paranet 70% and paranet 90% on peat moss significantly different from those on fern, husk charcoal, and cocopeat. Fern was not significantly different from husk charcoal, but significantly different from peat moss and cocopeat.

Table no 2: Results of Duncan's multiple range test on effects of treatments of different growing media at shade levels on plantlet height after acclimatization for 12 WAP

Treatment of	Media			
Shade level	Peat moss	Fern	Husk charcoal	Cocopeat
Paranet 50%	3.20 a B	2.32 a D	2.68 a C	4.23 a A
Paranet 70%	2.47 ab B	1.79 a C	1.97 b C	2.99 b A
Paranet 90%	1.53 b B	1.01 b C	1.23 c C	1.84 c A

Note: Numbers followed by the same letter were not significantly different according to the DMRT test at the 0.05 level. Capital letters are read horizontally (rows) and lowercase letters are read vertically (columns)

The greatest increase in plant height was found in cocopeat media (4.23 cm). This was because cocopeat has nutrients needed by plantlets and has porous texture so that the contact area of the root surface with the media was larger than that of fern and husk charcoal. Coconut fiber substrate and kadaka root can improve the Phalaenopsis Hybrid Plantlet root system, while sphagnum (peat moss) supports water availability so that plantlet fresh weight can be increased [20]. Cocopeat either alone or in combination with charcoal is the best acclimatization medium for orchids C. pandurata, C. asperata, and Oncidium linda isler x Odorais princess yH tween star [5].

Leaf Length

On the leaf length parameter, the interaction of treatments of shades on growing media had very significant effects. Table 3 shows that the effects of paranet 50% on peat moss were significantly different from those on fern, husk charcoal, and cocopeat. The growing medium of fern was not significantly different from husk charcoal, but significantly different from peat moss and cocopeat. The effects of paranet 70% and paranet 90% on peat moss were significantly different from those on fern and cocopeat, but not significantly different

from those on husk charcoal. Fern was significantly different from peat moss, husk charcoal and cocopeat. Cocopeat was significantly different from peat moss, fern, and husk charcoal. The least increase in leaf length was found in paranet 90% + fern. This was due to larger fern particles so that the surface area was relatively low and had a large cavity as a result of fewer roots attached to the medium.

Table no 3: Results of Duncan's multiple range test on effects of treatments of different growing media at shade levels on leaf length after acclimatization for 12 WAP

Treatment of	Media			
Shade level	Peat moss	Fern	Husk charcoal	Cocopeat
Paranet 50%	2.79 a B	2.20 a C	2.28 a C	3.21 a A
Paranet 70%	2.23 ab B	1.85 a C	2.17 a B	2.52 b A
Paranet 90%	1.53 b B	1.01 b C	1.99 a AB	2.34 b A

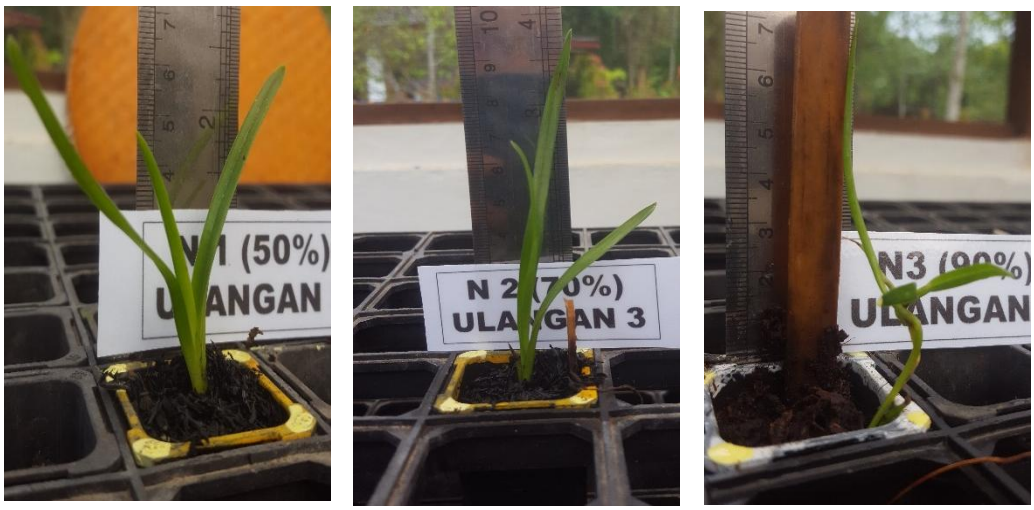
Note: Numbers followed by the same letter were not significantly different according to the DMRT test at the 0.05 level. Capital letters are read horizontally (rows) and lowercase letters are read vertically (columns).

Sand with a particle diameter of ± 1 mm has a relatively low surface area per gram and has a large space (channels between particles). Meanwhile, clay soil with a particle diameter of < 2 m has a larger surface area and a smaller space [21].

Number of Leaves

The effects of treatment interactions were not significantly different (p 0.289) on the number of leaves. This was due to variations in the formation of perfect leaves that took a relatively long time while the allocation of research time was limited. The increase in the number of leaves of the best acclimatized Doritaenopsis Plantlets was at moderate light levels, namely, PPFD 250 – 350 µmol m^{-2} s^{-1} compared to low light (150 – 200 µmol m^{-2} s^{-1}) and high (400 – 500 µmol m^{-2} s^{-1}) [11].

Figure no 1: Results of tiger orchid acclimatization at different shade levels (source: personal document)



The effect of independent shade was significantly different on the number of leaves. In (Figure 1) it can be seen that plantlets of tiger orchids on paranet 90% with the same medium, namely husk charcoal, experienced growth disorientation. It was due to the light intensity at paranet 90% only 52 µmol m^{-2} s^{-1}.

Survival Percentage

The effect of treatment interactions was significantly different on the survival percentage (*p* 0.015). This was due to the mechanism of the movement rate of water flow from the media to the plant and the movement of water inside the plant which was influenced by evaporation and leaf temperature. The movement of water flow from the soil/media to the plant and the movement of water within the plant is influenced by water evaporation and leaf temperature which results in negative pressure in the xylem [21].

Table no 4: Results of Duncan's multiple range test on effects of treatments of different growing media at shade levels on survival percentage after acclimatization for 12 WAP

Treatment of	Media			
Shade level	Peat moss	Fern	Husk charcoal	Cocopeat
Paranet 50%	2.79 a B	2.20 a C	2.28 a C	3.21 a A
Paranet 70%	2.23 ab B	1.85 a C	2.17 a B	2.52 b A
Paranet 90%	1.53 b B	1.01 b C	1.99 a AB	2.34 b A

Note: Numbers followed by the same letter were not significantly different according to the DMRT test at the 0.05 level. Capital letters are read horizontally (rows) and lowercase letters are read vertically (columns).

The results of Duncan's multiple range test (Table 4) show that only the combination of paranet 90% + husk charcoal was different. This is because husk charcoal has a rough texture so that relatively few root surfaces can stick to the media and husk charcoal has a light weight so it is not able to keep the plant upright. Even though husk charcoal has a porous texture, it can still retain nutrient fluids well [17].

Plantlet Vigor

The standard for the vigor assessment in the acclimatization phase of tiger orchids is seemingly not available yet. For the rice plants, there is a Guidebook for Rice Plant Characterization and Evaluation System (DEPARTMENT OF AGRICULTURE, 2003). Departing from this guide, for tiger orchids, the author describes vigor as the ability of tiger orchids to avoid light and drought stress with indicators of epidermal thickness, cuticle thickness, and leaf color.

The results of measurements of the thickness of the epidermis and cuticle can be seen in (Figure 2). The thickest epidermal thickness was found in the treatment combination of the shade (n1) paranet 50% + medium (m1) peat moss 13.50 µm, the thinnest epidermal thickness was found in the treatment combination of the shade (n3) paranet 90% + medium (m2) fern 9.78 µm. The thickest cuticle thickness was found in the treatment combination of the shade (n1) paranet 50% + medium (m1) peat moss 3.15 µm and the lowest cuticle thickness was found in the treatment combination of the shade (n3) paranet 90% + medium (m2) fern 1.64 µm. This is in accordance with the results of research by Jeon, et al. (2005), the formation of wax on the leaf surface of Doritaenopsis plants is more at high light intensity.

Figure no 2: Graph of measurement for the thickness of epidermis and cuticle of tiger orchid leaves after acclimatization for 12 WST

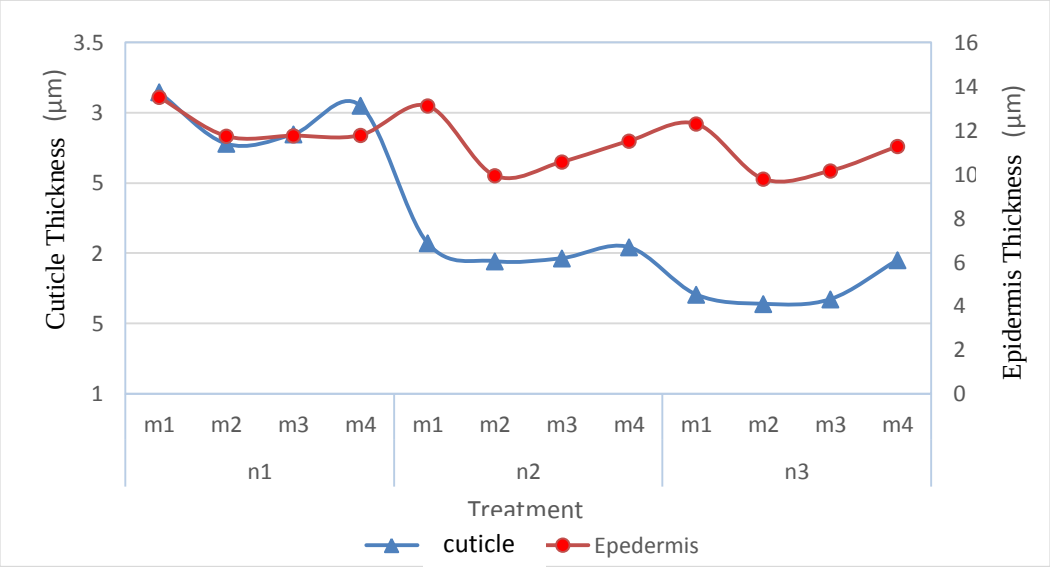


Table no 5: Scores of cuticle thickness, epidermis thickness, and leaf color

Category	Cuticle thickness (µm)	Epidermis thickness (µm)	Leaf color*)
1	≤ 1.58	≤ 9.61	RHS 143A
2	1.58 – 2.89	9.61 – 13.27	RHS 143C
3	≥ 2.89	≥ 13.27	RHS 144A

* Color is based on *color charts royal horticultural society (RHS color charts)*

The recapitulation of the results of the plantlet vigor score shown in (Table 6) shows very good vigor. There is only 1 plantlet, namely in the combination of paranet 50% + peat moss treatment (cuticle thickness 3.18 µm, epidermis 13.53 µm, leaf color RHS 144A). This is because the intensity of light in accordance with the light intensity needs of tiger orchids and peat moss can provide sufficient water and nutrients for growth. According to Jeon, et al. (2005) the best light level for acclimatization of Doritaenopsis plantlets is at the medium light level, PPFD 250 – 350 µmol m^{-2} s^{-1} compared to the low light level (150 – 200 µmol m^{-2} s^{-1}) and the high light level (400 – 500 µmol m^{-2} s^{-1}). At the enlargement stage the best media for the growth of Phalaenopsis orchid seedlings is moss combined with brick or charcoal, and then fern combined with brick or charcoal [22].

Table no 6: Score recapitulation of plantlet vigor

Category	Score	Treatment	Number
Very low	≤ 3.69	n3m3.2,n3m1.3,n3m2.3	3
Low	3.69 – 5.25	n3m1.1, n3m2.1, n3m3.1, n3m4.1, n3m2.2, n3m3.3, n3m4.3	7
Medium	5.25 – 6.81	n1m2.3, n2m2.1, n2m4.1, n2m1.2, n2m2.2, n2m3.2, n2m4.2, n2m1.3, n2m2,3, n2m3.3, n2m4.3, n3m1.2, n3m4.2	13
Good	6.81 – 8.36	n1m1.1, n1m2.1, n1m3.1, n1m4.1, n1m2.2, n1m3.2, n1m4.2, n1m1.3, n1m3.3, n1m4.3, n2m1.1, n2m3.1	12
Very good	≥ 8.36	n1m1.2	1

IV. Conclusion

There were effects of treatment interaction of growing media at shade levels on the growth and vigor of tiger orchid plantlets during acclimatization, and there were effects of shade levels and growing media independently on the growth and vigor of tiger orchid plantlets during acclimatization.

The best treatment combination for growth was the combination of paranet 50% + coopeat (plant height = 4.23 cm, leaf length = 3.21 cm, survival percentage = 100%) and the best treatment combination for plantlet vigor was the combination of paranet 50% + peat moss. The use of conversion tools / calculator needs to be tested further in order to obtain the right correction factor to measure photosynthetic photon flux densities so as to facilitate the application in the field.

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