



***In-Vitro* Growth of Vanda Tricolor Orchid Plantlets of Suavis Variety on ZPT IBA and Activated Charcoal Treatments**

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ABSTRACT

The aim of this research was to examine the effect of ZPT IBA and activated charcoal on plantula growth of *Vanda tricolor* orchid *suavis* variety on MS basic media, and to obtain the best concentration of IBA and activated charcoal from MS basic media treatment for plantula growth on *Vanda tricolor* orchid *suavis* variety. This research was done in the Tissue Culture Laboratory, Faculty of Agriculture Faculty, 17 Agustus 1945 Samarinda University from February until April 2019. This research used factorial experiment 4x4 which arranged in Randomized Complete Design. The Factor of ZPT IBA (I) which were consist of 4 levels, namely without ZPT IBA (i0); 0,3 ppm L⁻¹ media (i1); 0,6 ppm L⁻¹ media (i2), and 0,9 ppm L⁻¹ media (i3). The factor of activated charcoal (A) consisting of 4 levels: without activated charcoal (a0); 2 g L⁻¹ media (a1); 4 g L⁻¹ media (a2); and 6 g L⁻¹ media (a3). The stages of research activities are as follows: (1) preparation of plant materials, (2) preparation of tools, (3) sterilization of equipment; (4) making media stock; (5) preparation of treatment media (in the form of MSO media as a control and media treated with IBA and activated charcoal); (6) plantula planting; (7) plantula maintenance, (8) data collection and analysis, and (9) reporting. The result a of research showed that : (1) the factor of IBA growth regulator affected significantly on root length at 3 months after planting, but no affected significantly on the increase in height and number of leaves at 1, 2 and 3 months, and the increase in the number of roots at age 3 months after planting the *Vanda tricolor* Orchid plantlet Varieties *Suavis*; (2) the factor of activated charcoal affected significantly on the increase in height 1 month after planting, but no affected significant on the increase in plant height 2 and 3 months, the increase in the number of leaves 1.2 and 3 months, the increase in the number of roots and length roots at the age of 3 months after planting the plantlet *Vanda tricolor* Orchid *suavis* variety; and (3) there was an interaction between ZPT IBA and activated charcoal treatment in the increase in height at 2 months, the number of leaves at 3 months, and the increase in the number of roots at 3 months after planting, but there was no interaction between the two treatment factors on increase in height at 1 and 3 months, increase in the number of leaves at 1 and 2 months, and increase in root length at 3 months after planting plantlet *Vanda tricolor* Orchid *Suavis* variety.

Keywords: IBA, Activated Charcoal, Plantlet, *Vanda tricolor*

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INTRODUCTION

Orchid flowers are one of the commodities that have high economic value, so that many business people seek them as a source of income. The *Vanda tricolor* orchid *suavis* variety is a type of orchid that is praised by many people because of the beauty of its flowers. This is because the color of the *Vanda tricolor* orchid flower *suavis* variety is very diverse and the flowers last a long time [1-2].

Orchid propagation using vegetative and generative methods often encounters obstacles, namely the difficulty of obtaining large quantities of seeds in a short time, free from pests and diseases and high production. To overcome these obstacles is the tissue culture. Therefore, it is necessary to seek technological improvements to reproduce and regenerate the *Vanda tricolor* orchid. Alternative propagation techniques that can be used is through in vitro culture.

In vitro culture is a technique of isolating plant parts, growing them in artificial media containing complete nutrition in a sterile environment so that the plant parts grow into perfect plants [3, 6]. Propagation of orchids in vitro using vegetative parts as explants such as leaves or shoots can produce protocorm like bodies or plantlets that have the same characteristics as the parent. The success of in vitro orchid propagation is determined by many things, including the composition of the media used [12].

The growth plant pattern in a tissue culture system is determined by the net physiological condition of the

plant concerned due to the influence of internal and environmental conditions. Environmental conditions such as light, water and nutrient supply as well as giving growth regulators can be modified in such a way as to control the physiological conditions of the explants [13].

Growth regulators (ZPT) are non-nutritional organic compounds which in low concentrations (<1 mM) can encourage, inhibit or qualitatively change plant growth and development. BAP is a group of cytokinins that function in cell division. Its most important role is to induce callus formation, stimulate bud formation and break cell dormancy [5].

Growth regulators (PGR) which play an important role in tissue culture are auxins and cytokinins. ZPT which includes auxins are IBA (Indole Butyric Acid) and NAA (Naphthyl Acetic Acid), examples of cytokinins are BAP (Benzyl Amino Purin) and kinetin. IBA and NAA are active compounds in small amounts which are synthesized in certain parts and are generally translocated to other parts of the plant where these substances cause a response in root growth [11].

The use of activated charcoal is also an important factor in planting vanda orchids. Activated charcoal can bind molecules, both organic and inorganic in the culture medium (Mattson and Mark Jr, 1981), activated charcoal has been used in various micropropagation systems, has been reported to stimulate embryogenesis [13].

The aims of the research were to examine the effect of ZPT IBA and activated charcoal on plantula growth of *Vanda tricolor* orchid suavis variety on MS basic media, and to obtain the best concentration of IBA and activated charcoal from MS basic media treatment for plantula growth on *Vanda tricolor* orchid suavis variety.

MATERIAL AND METHODS

A. Place and Time

This research was done in the Tissue Culture Laboratory, Faculty of Agriculture Faculty, 17 Agustus 1945 Samarinda University from February until April 2019.

B. Materials and Tools

The material used is the *Vanda tricolor* orchid plantula suavis variety. ZPT IBA and activated charcoal, chemicals for MS basic media (macro, micro nutrients, CaCl₂, FeSO₄, Vitamins and myo-inositol) sterile aquades, spiritus, sucrose, agar powder, 70 and 95% alcohol. The equipment used is laminar air flow, autoclave, analytical balance, Erlenmeyer, measuring cup, beaker, petri dish, pipette, scalpel, tweezers, lamp, bugsen, culture bottle, sprayer, ruler, camera and stationery.

C. Research Design

This research used factorial experiment 4x4 which arranged in Randomized Complete Design was repeated 3 times. The factor is ZPT IBA (I) consists of 4 levels, namely without ZPT IBA (i0); 0,3 ppm L⁻¹ media (i1); 0,6 ppm L⁻¹ media (i2), and 0,9 ppm L⁻¹ media (i3). The factor is activated charcoal (A) consisting of 4 levels: without activated charcoal (a0); 2 g L⁻¹ media (a1); 4 g L⁻¹ media (a2); and 6 g L⁻¹ media (a3).

D. Research Procedures

The stages of research activities are as follows: (1) preparation of plant materials, (2) preparation of tools, (3) sterilization of equipment; (4) making media stock; (5) preparation of treatment media (in the form of MSO media as a control and media treated with IBA and activated charcoal); (6) plantula planting; (7) plantula maintenance, (8) data collection and analysis, and (9) reporting.

E. Data collection

The data collected were: increase in plantlet height and number of plantlet leaves at 1, 2, and 3 months after planting increase in the number of roots and length of roots aged 3 months after planting.

F. Analysis Data

Data analysis was carried out using Analysis of Variance and Least Significant Difference test (LSD) at 5% level.

RESULTS AND DISCUSSION

A. The Effect of IBA

The results of variance showed that the ZPT IBA (I) treatment had no affected significantly on the increase in height and number of leaf plantlets aged 1.2 and 3 months after planting, and the increase in the number of roots aged 3 months after planting, but had a affected significantly on the increase in root length at age 3 months after planting. The Data of the research are presented in Table 1.

Table 1. Research Data of the ZPT IBA Effect on the Growth of *Vanda tricolor* Orchid Plantlet Suavis Variety

Treatment Factor	Height Increment (cm)		
	1 BST	2 BST	3 BST
Treatment of ZPT IBA (I)	tn	tn	tn
No ZPT IBA (i0)	1,75	2,42	2,25
0,3 ppm (i1)	2,17	2,00	2,00
0,6 ppm (i2)	3,67	2,67	2,17
0,9 ppm (i3)	2,08	3,58	1,58
Treatment Factor	Leave Increment		
	1 BST	2 BST	3 BST
Treatment of ZPT IBA (I)	tn	tn	tn
No ZPT IBA (i0)	1,42	1,17	1,17
0,3 ppm (i1)	1,75	1,42	1,17
0,6 ppm (i2)	1,58	1,25	1,50
0,9 ppm (i3)	1,42	1,08	1,25

Treatment of ZPT IBA (I)	Increase in Number of Roots at 3 BST	Increase in Root Length at 3 BST (cm)
		tn
No ZPT IBA (i0)	1,33	2,33 b
0,3 ppm (i1)	1,25	3,25 a
0,6 ppm (i2)	1,08	2,50 b
0,9 ppm (i3)	1,50	3,83 a

Note: The mean score followed by the same letter is different not based on the advanced test with BNT level 5%. tn = no significant; * = affected significantly; ** = affected very significantly, BST = months after planting

In Table 1 show that giving ZPT IBA 0.3 – 0.9 ppm resulted in an increase in height and number of leaves at the age of 1, 2 and 3 months after planting, the number and length of roots were 3 months after planting the Orchid Plantlet *Vanda tricolor* suavis variety which varies, which can increase height at the age of 1 and 2 months, number of leaves at the age of 3 months after planting and root length at the age of 3 months after planting. However, in general, giving ZPT IBA can stimulate the growth of root length. As reported by Shrestha [9] that MS media added 2 ppm IBA was the most effective condition for rooting of *Coelogyne ovalis* Lindl. The reported by Aktar et al. [1] that application of 1 mg L⁻¹ IBA resulted in 1.81 root formation per explant in *Dendrobium* sp. Furthermore, reported by Talukder et. al. [10] that the application of 2 mg L⁻¹ IBA on MS media resulted in 1.62 roots per plantlet *Dendrobium* sp.

B. Effects of Activated Charcoal

The analysis of variance showed that the activated charcoal treatment (A) no affected significantly on the increase in height at 2 and 3 months, the increase in the number of plantlet leaves at 1.2 and 3 months, the increase in the number of roots and root length at 3 months after planting, but had a affected significantly on the increase in height at the age of 1 month after planting. The Data of the research are presented in Table 2.

Table 2. Research Data of the Activated Charcoal Effect on the Growth of *Vanda tricolor* Orchid Plantlet suavis variety

Treatment Factor	Height Increment (cm)		
	1 BST	2 BST	3 BST
Activated Charcoal Treatment (A)	*	tn	tn
No Activated Charcoal (a0)	1,92 b	2,58	2,75
2 g L ⁻¹ media (a1)	3,33 a	2,83	1,25
4 g L ⁻¹ media (a2)	2,25 b	2,83	1,67
6 g L ⁻¹ media (a3)	2,17 b	2,42	2,33
Treatment Factor	Leave Increment		
	1 BST	2 BST	3 BST
Activated Charcoal Treatment (A)	tn	tn	tn
No Activated Charcoal (a0)	1,42	1,17	1,17
2 g L ⁻¹ media (a1)	1,75	1,42	1,17
4 g L ⁻¹ media (a2)	1,58	1,25	1,50
6 g L ⁻¹ media (a3)	1,42	1,08	1,25

Treatment Factor	Increase in Number of Roots at 3 BST	Increase in Root Length at 3 BST (cm)
Activated Charcoal Treatment (A)	tn	tn
No Activated Charcoal (a0)	1,25	2,17
2 g L ⁻¹ media (a1)	1,17	2,75
4 g L ⁻¹ media (a2)	1,42	2,50
6 g L ⁻¹ media (a3)	1,33	4.50

Note: The mean score followed by the same letter is different not based on the advanced test with BNT level 5%. tn = no significant; * = affected significantly; ** = affected very significantly, BST = months after planting

Data of research presented in Table 2 show that the treatment of activated charcoal (2, 4, and 6 g L⁻¹ media) tends to result in a greater increase growth of the of *Vanda tricolor* Orchid Plantlet suavis variety compared to treatment without no activated charcoal (a0). Giving 4 - 6 g L⁻¹ media can encourage an increase in the growth leaves, increase in the number of roots and length of root. Thus it can be stated that in general the addition of activated charcoal to MS media gives a better average growth value than without the addition of activated charcoal. The results of a study reported by [11], application of activated charcoal at a dose of 2 g L⁻¹ into MS media can increase the growth of plantlet height, leaf area, number of tiller shoots, and number of roots. It is suspected that the application of activated charcoal with a certain concentration in MS media has a good effect. According to Reserachers stated that this activated charcoal plays an important role in seed germination and flowering of orchids, stimulating rooting, tuber formation, stem elongation, provision of nutrients, absorption of vitamins, metal ions and growth regulators, as well as inhibition of changes and darkening of the culture media. The addition of 2 g L⁻¹ to in vitro culture media resulted in a higher germination percentage of up to 18.12% [8] and leaf length and width of up to 55 and 65% respectively compared without the addition of activated charcoal on *Dendrobium* orchids [3].

C. Effect of Interaction between ZPT IBA and Activated Charcoal

The analysis of variance results showed that interaction between ZPT IBA and activated charcoal treatment on the increase in height at 2 months, the number of leaves at 3 months, and the increase in the number of roots at 3 months, but there was no interaction on increase in height at 1 and 3 months, increase in the number of leaves at 1 and 2 months, and increase in length of at 3 months after planting the plantlet *Vanda tricolor* Orchid Suavis Variety. These conditions indicate that the ZPT IBA factor and the activated charcoal factor can either jointly or individually influence the growth of the *Vanda tricolor* Orchid variety Suavis plantlets.

The results of the research on the effect of the interaction between ZPT IBA and activated charcoal treatment on the increase in height and number of leaves aged 1, 2 and 3 months, number of roots and root length aged 3 months after planting the plantlet *Vanda tricolor* Orchid variety Suavis are presented in Table 3.

Table 3. Research Data of the Effect of Interaction between ZPT IBA and Activated Charcoal on the Growth of Orchid Plantlets of *Vanda tricolor* Suavis Variety

Treatment Factor	Height Increment (cm)		
	1 BST	2 BST	3 BST
Interaction IxA	tn	*	tn
i0a0	1,33	2,67 a	2,00
i0a1	2,00	1,67 b	1,33
i0a2	1,67	3,67 a	3,00
i0a3	2,00	1,67 b	2,67
i1a0	1,00	2,00 b	2,33
i1a1	4,00	1,00 c	1,33
i1a2	2,00	1,33 c	1,00
i1a3	1,67	3,67 a	3,33
i2a0	2,67	1,33 c	4,00
i2a1	5,67	6,00 a	1,33
i2a2	2,33	1,33 c	1,33
i2a3	4,00	2,00 b	2,00
i3a0	2,67	4,33 a	2,67
i3a1	1,67	2,67 a	1,00
i3a2	3,00	5,00 a	1,33
i3a3	1,00	2,33 b	1,33
Treatment Factor	Leave Increment		
	1 BST	2 BST	3 BST

Interaction IxA	tn	tn	*
i0a0	1,33	1,00	1,00 c
i0a1	1,67	2,00	1,33 b
i0a2	2,00	1,33	2,67 a
i0a3	2,33	1,00	1,33 b
i1a0	1,00	1,33	1,00 c
i1a1	1,33	1,00	1,00 c
i1a2	1,67	1,33	1,33 b
i1a3	1,33	1,33	1,33 b
i2a0	1,67	1,33	1,33 b
i2a1	2,00	1,33	1,33 b
i2a2	1,33	1,00	1,00 c
i2a3	1,00	1,00	1,00 c
i3a0	1,67	1,00	1,33 b
i3a1	2,00	1,33	1,00 c
i3a2	1,33	1,33	1,00 c
i3a3	1,00	1,00	1,33 b

Treatment Factor	Increase in Number of Roots at 3 BST	Increase in Root Length at 3 BST (cm)
Interaction IxA	*	tn
i0a0	1,33 c	1,00
i0a1	1,00 d	1,33
i0a2	2,00 a	2,33
i0a3	1,00 d	4,46
i1a0	1,00 d	1,33
i1a1	1,00 d	3,33
i1a2	1,67 b	3,00
i1a3	1,33 c	5,33
i2a0	1,00 d	4,00
i2a1	1,33 c	1,33
i2a2	1,00 d	1,67
i2a3	1,00 d	3,00
i3a0	1,67 b	2,33
i3a1	1,33 c	5,00
i3a2	1,00 d	3,00
i3a3	2,00 a	5,00

Note: The mean score followed by the same letter is different not based on the advanced test with BNT level 5%. tn = no significant; * = affected significantly; ** = affected very significantly, BST = months after planting

The treatment interactions showed significant results, in the vegetative growth phase, where it was suspected that the administration of growth regulators and activated charcoal at different doses would give different growth. As Pierik [7] stated that in general, auxin increases cell elongation, cell division, and the formation of adventitious roots. Meanwhile, low concentrations of auxin will increase the formation of adventitious roots, and auxin with high concentrations will stimulate callus formation and suppress morphogenesis [9]. And as in tissue culture techniques, the presence of growth regulators has a very real effect. The application of activated charcoal can increase molecules, both organic and inorganic in culture media, this compound has been used in various system of micropropagation. The exact effect of activated charcoal is still unknown, there are several operating modes that may occur, and activated charcoal can regulate the supply of certain endogenous growth substances [8].

CONCLUSIONS AND RECOMMENDATIONS

A. Conclusion

1. The application of IBA growth regulators had a affected significantly on root length 3 months after planting but has no affected significantly on the increase in height and number of leaves at 1, 2 and 3 months, and the increase in the roots number 3 months after planting the *Vanda tricolor* Orchid plantlet, Suavis variety.
2. The applicaton of activated charcoal has a affected significantly on the increase in height 1 month, but has no affected significantly on the increase in plant height 2 and 3 months, the increase in the number of leaves 1.2 and 3 months, the increase in the roots number and root length at the age of 3 months after

planting the *Vanda tricolor* Orchid plantlet of the Suavis variety.

3. There was an interaction between the ZPT IBA and activated charcoal treatment in the height increase at 2 months, the leaves number at 3 months, and the increase in the roots number at 3 months after planting the plantlet *Vanda tricolor* Orchid, Suavis variety.

B. Suggestion

1. For the propagation of the *Vanda Tricolor* Orchid plant of the Suavis variety, it is recommended to use the right treatment of IBA (without IBA growth regulators and with 4 g L⁻¹ media of activated charcoal).
2. Keeping the environment sterile because it can reduce contamination caused by microorganisms that live in the air.

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