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Propagation Of The *Vanda helvola* Orchid In Vitro

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Abstract: Orchids are more economical and tall than plants and other decorations. One of the orchids cultivated is the *Vanda* orchid. Seed orchid size is small and does not have endosperm, so it is not easy to germinate in nature, so tissue culture is one _ multiplication orchid in amount a lot and a short time. This study aims to know the growth of the seed orchid *Vanda helvola* on different media for 12 days after plant. Seed orchid *V. helvola* grown on modified Murashinge and Shoog (MS), modified Vaccin and Wend (VW) media and organic media. The research was conducted at the Tissue Culture Laboratory of the UPT Center for Food Crops and Horticulture (BBIPTH), Luwus Tanaban, from February to May 2023. The research method used was observation and experimentation, and the research design used RAK (group random design). Research shows modified MS Media, modified VW and organic capable change seeds orchid *V. helvola* . Modified MS and modified VW media can induce seed orchid *V. helvola* germination, and MS media can induce formation protocol.

Keywords: Media; Protocorm; *Vanda helvola*.

Introduction

Orchids have unique colours, shapes, and characteristics, giving them a higher economic value than other ornamental plants. One of the orchids widely cultivated is the orchid from the genus *Vanda*. Orchids have small seeds and do not have endosperm, which makes it difficult for orchids to germinate in nature [1].

Orchid propagation is mostly done by tissue culture. The plant tissue isolation method has been carried out in several species of orchids with different growth media [2]. Propagation plants using tissue culture capable of producing new plants inside amount Lots in a short time [3]

Media use in tissue culture is very diverse and varies according to needs. Media modifications are carried out to improve tissue culture results, such as adding growth regulators and organic materials. The research results of Ambarwati [4] showed that the addition of organic materials in the form of coconut water, potato extract, tomato extract and banana extract separately to VW media affected the growth of the *Dendrobium sp., Oncidium sp., and Phalaenopsis sp*. Based on the research results of Nisa [5], adding 150 ml/L coconut water to VW media affects root formation and best root length in *Dendrobium sp* orchids. The research results of Maulidia [6] growth of *Dendrobium* orchids singkawangense on ½ MS media with adding tomato extract at various concentrations can provide the same good growth as full MS media. Orchid germination media commonly used are Vaccin and Went (VW), Knudson C Murashinge and Skoog (MS) [7-8].

The tissue culture medium consists of essential amino acids, inorganic salts, vitamins, buffer solutions, and an energy source, usually glucose. This medium is very important for evaluating the success of plant propagation in vitro. Therefore, the right and appropriate dose is needed to make media to maximize the results. Two types of media, solid and liquid media, can be used to grow PLBs (photochrom-like bodies) until the formation of plantlets. Conversely, liquid media can be used to grow explants until the formation of PLB, which is an explant that will grow tissue such as a white callus [11-12].

The purpose of this research was to find out how to propagate orchid plants by seed stocking techniques using MS (Murashinge and Skoog) media, VW (Vaccine and Went) media and OR (Organic) media conducted at the UPT Tissue Culture Laboratory of the Food and Horticulture Main Seed Center (BBIPTH), Luwus Tanaban.

Research Methods

The research was conducted at the Tissue Culture Laboratory of the UPT Center for Food Crops and Horticulture (BBIPTH), Luwus Tanaban, from February to May 2023. The research method used is observation and experimentation, and the research design uses RAK (group random design).

Media Creation

The media used are modified MS, VW, and organic.VW and MS media begin with making stock solutions based on their respective compositions (Appendix 1), which consist of macro, micronutrients and vitamins.

Media Murashinge and Skoog (MS)

The macro, micro and vitamin nutrient stock solution is put into a glass beaker. 100 g of tomatoes have been blended, and 20 g of sugar added to the beaker glass. Water was added until the solution reached a volume of 1000 mL while stirring. The pH is adjusted between 5.6 – 5.8 by adding NaOH or HCl. The media was heated while adding 8 g of agar. The medium is heated until it boils. The media was

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poured into 30 ml bottles and sterilized using an autoclave at a temperature of 121 ° C and a pressure of 1.5 atm for 30 minutes.

Media Vaccine and Went (VW)

The macro, micro and vitamin nutrient solutions are put into a glass beaker. Blended 150 g ambon banana, 150 ml coconut water, 20 g granulated sugar, 2 g activated charcoal, 2 g gandasil D and 3 ml fish emulsion put into a beaker. Water was added until the solution reached a volume of 1000 mL. The pH is adjusted between 5.6 – 5.8 by adding NaOH or HCl. The media was heated while adding 8 g of agar. The medium is heated until it boils. The media was poured into 30 ml bottles and sterilized using an autoclave at a temperature of 121 ° C and a pressure of 1.5 atm for 30 minutes.

Organic Media

250 g potatoes peeled and cut into cubes. Please put it in a pan containing 500 ml of water and boil until it boils. The boiled potatoes are filtered and put into a beaker. Blended 150 g Ambon banana, 150 mL coconut water, 20 g granulated sugar, 2 g gandasil D, 200 mg vitamin C and 3 mL fish emulsion put into a beaker. Water was added until the solution reached a volume of 1000 mL. The pH is adjusted between 5.6 – 5.8 by adding NaOH or HCl. The media was heated while adding 8 g of agar. The medium is heated until it boils. The media was poured into 30 ml bottles and sterilized using an autoclave at a temperature of 121 ° C and a pressure of 1.5 atm for 30 minutes.

Table 1. Composition and Consentration of Media

Composition	Concentration
Media Vaccine and Went (VW)	
Macro Elements	
$Ca_3(PO_4)_2$	200 mg/L
KNO_3	525 mg/L
KH_2PO_4	250 mg/L
$MgSO_4.7H_2O$	250 mg/L
$(NH_4)_2SO_4$	500 mg/L
Micro Elements	
$MnSO_4.4H_2O$	7.5 mg/L
Fe tatrate	28 mg/L
Fe Sulfate	27.8 mg/L
Na2 EDTA _	37.2 mg/L
Vitamin	
Myoinositol	100 mg/L
Pyridoxine	0.5 mg/L
Niacin HCl	0.5 mg/L
Thiamine	0.5 mg/L
Media Murashinge and Skoog (MS)	
Macro Elements	
$CaCl_2.2H_2O$	440 mg/L
KNO_3	1900 mg/L
KH_2PO_4	170 mg/L
$MgSO_4.7H_2O$	370 mg/L
NH_4NO_3	1650 mg/L
Micro Elements	
$FeSO_4.7H_2O$	27.8 mg/L
Na2 EDTA _	37.3 mg/L
$MnSO_4.4H_2O$	22.3 mg/L
$ZnSO_4.7H_2O$	8.6 mg/L
H_3BO_3	6.2 mg/L
KI	0.83 mg/L
$CuSO_4.5H_2O$	0.025 mg/L
$Na_2MoO_4.2H_2O$	0.25 mg/L
$CoCl_2.6H_2O$	0.025 mg/L
Vitamin	
Myoinositol	100 mg/L
Pyridocin	0.5 mg/L
Niacin HCl	0.5 mg/L
Thiamine	0.5 mg/L

Sterilization and Planting of Explants

Orchid capsules are washed using detergent (mama lemon), soaked in 10% NaClO (bayclin) solution for 20 minutes, and then soaked in 2% fungicide solution for 20 minutes. In the Laminar Air Flow Cabinet (LAFC), the orchid capsule is placed on a petri dish and split with scissors. Seed orchids were taken using _ tweezers and planted in the media.

Design Study

Data obtained in this research is analyzed qualitatively and quantitatively. Observed parameters that change seed orchids and their formation of protocorm-like bodies (plb).

Results and Discussion

Growth Time And Changes in *Vanda helvola* Seeds

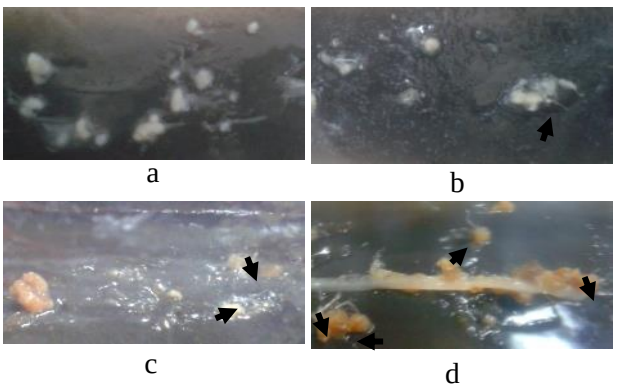

Figure 1. Orchid seeds *V. helvola* grown on organic media: (a) newly planted, (b) 3 WAP, (c) 9 WAP and (d) 12 WAP
➡ : a growth resembling cotton

V. Helvola orchid seeds during 12 weeks after planting (WAP), on MS, VW and organic media, the change response occurred at the same time, namely 3 WAP. The visible growth response is a growth that resembles cotton on VW (Figure 1) and organic (Figure 2) media. Growth resembling cotton on VW and organic media forms from 3 WAP to 12 WAP. In organic media, cotton-like growth

appeared relatively later than in VW media. On MS media, planted orchid seeds began to swell at three WAP, orchid seeds turned slightly yellowish at 4 WAP, yellowish green at 6 WAP, green at 8 WAP and prototoms began forming at 9 WAP. More and more protocols appeared at 12 WAP in dark green (figure 3).

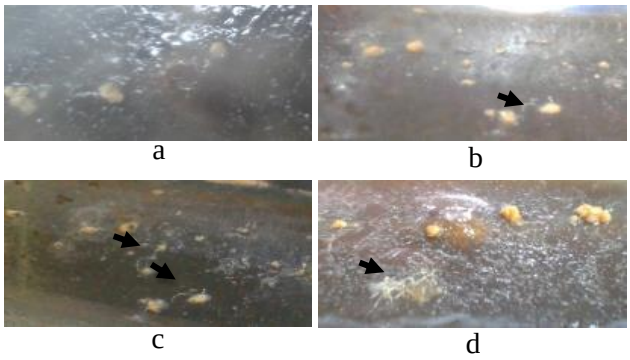

Figure 2. Development of orchid seeds *V. helvola* on VW media: (a) just planted. (b) 3 WAP, (c) 9 WAP, (d) 12 WAP
➡ : resembling growth _ cotton

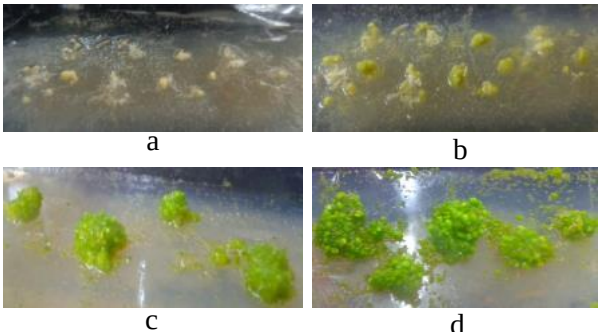

Figure 3. Development seed orchid *V. helvola* on MS media: (a) 3 WAP, (b) 6 WAP, (c) 9 WAP, (d) 12 WAP

Growth *Vanda helvola* Orchid

Seeds that show growth

At 12 WEST, the percentage of seeds that showed response growth highest, namely on MS (Vaccine and Went) media, which was 55.17%, and the percentage of the lowest was in VW (Murashinge and Skoog) media, namely 32.5%. And OR (Organic) media is 35,71^

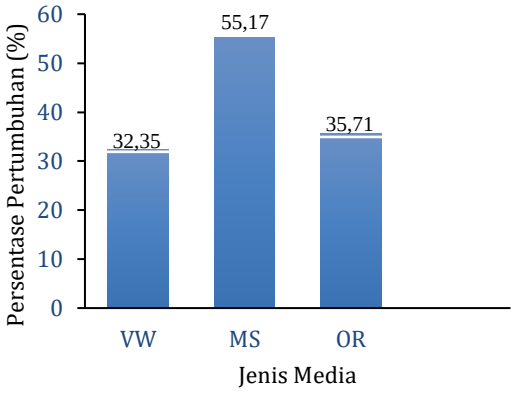

Figure 4. Percentage of growth of *Vanda helvola* orchid seeds

Stages of embryonic growth of *Vanda helvola* orchid seeds

The orchid seeds that had been planted were then observed under a microscope to determine the development of the embryo seeds in the three media. According to [11], orchid seed embryo development stages are divided into 6, namely stadium 1 (embryo in the orchid seed before planting), stage 2 (swollen embryo, still with testa), stage 3 (testa detached, embryo develops into a round white protocorm), s stadium 4 (protocorm turns yellow, round shape), stage 5 (green protocorm, round shape), stage 6 (green protocorm, elongated shape, Shoot Apical Meristem /SAM appears).

V. helvola seed embryos on VW media reached stage 4; namely, the prototom changed color to yellow, oval in shape. However, there were orchid seeds with swollen embryos, yellow and oval in shape. However, the testa was still attached to the tip of the orchid embryo (picture 5), so orchid seeds that experience this are included in stage 2. The dominant stage of orchid seed development on VW media is stage 3. The orchid embryo swells with a blackish-yellow colour and is oval in shape.

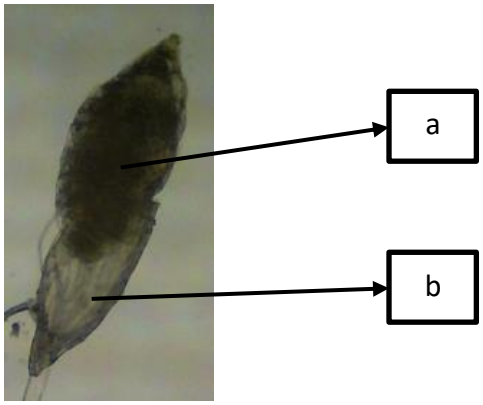

Figure 5. The testa is still attached to the orchid embryo on VW media. a). Swollen embryo b) The testa is almost separated from the embryo

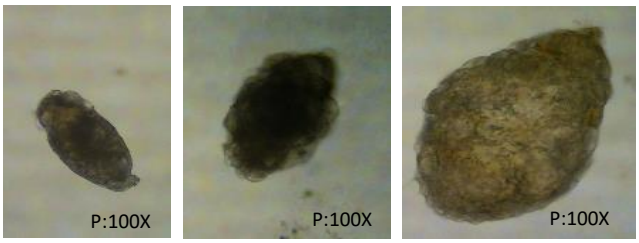

Figure 6. Development of seed embryos on VW media: (a) Stage 2, (b) stage 3, (c) stage 4

In organic media, the development stage of orchid embryos reaches stage 2; namely, the embryo is swollen and still has a testa; however, in some orchid seeds, orchid embryos were found that were swollen and yellow in color with the tip of the embryo having a testa that was almost separated from the embryo (figure 7). The dominant stage of embryo development in organic media is stage 2. The embryo is swollen and still has a testa.

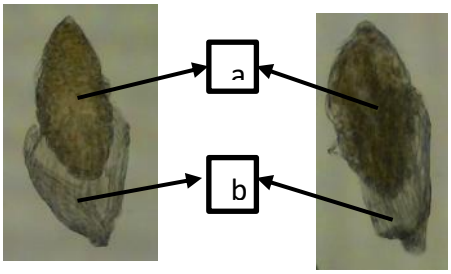

Figure 7. Testa is still attached to orchid embryos on organic media. a.) Swollen embryo b.) The testa is almost separated from the embryo

Figure 8. Development of stage 2 orchid seed embryos on organic media

On MS media, the development stage of the embryo reaches stage 6, with the dominant stage being stage 3. The testa detaches, and the embryo swells and takes an oval shape.

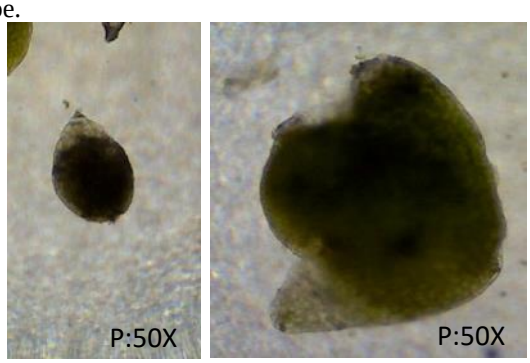

Figure 9. Orchid seed embryo development *V. helvola* : (a) stage 3, (b) stage 6

***Vanda helvola* orchid seeds which form prototoms**

The medium that can induce the growth of protolomes in *V. helvola* orchid seeds is MS media, with the percentage of seeds forming protolomes namely 24.14%. In contrast, VW and organic media are not able to form protolomes.

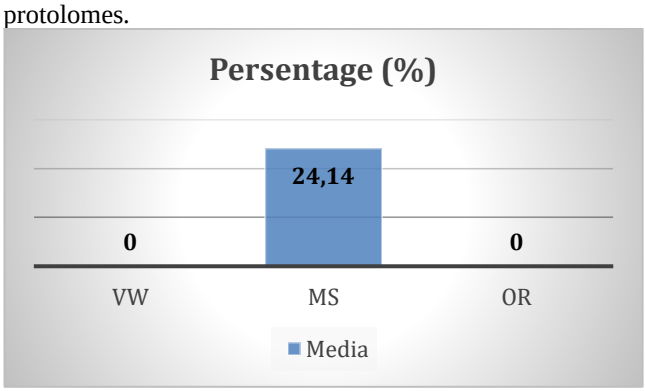

Figure 10. Percentage of seeds forming prototoms

The results showed that *Vanda helvola* orchid seeds could grow in the three media with different growth phases. Different media and organic materials added to the media influence the growth of *V. helvola* orchid seeds. Germination and growth of orchids are influenced by several factors, namely temperature, light, agar, minerals, sugar, pH, vitamins, growth regulators, complex compounds (coconut water, banana juice, peptone, etc.), activated charcoal, fruit age, endogenous hormone content. in seeds and media types [12-13].

Based on observations, *Vanda helvola* orchid seeds planted on MS and VW media could germinate, whereas orchid seeds planted on organic media could not germinate. Orchid seeds planted in VW media have a low growth response (figure 4) compared to organic media; however VW media can induce orchid seed embryo development to reach stage 4, whereas in organic media, orchid embryo development reaches stage 2, so it can be said that VW media able to induce germination of *V. helvola* orchid seeds. This follows Arditti's [2] statement that orchid seeds are said to germinate when the seeds swell, and the testa is completely separated from the embryo, namely at stage 3.

Imbibition is the process of water entering from outside the seed into the seed to increase the seed's water content, which causes the seed to germinate [14]. According to Kurniati [15], imbibition in Dendrobium capra JJ Smith orchid seeds occurs when the seeds experience testa swelling, which causes the testa to rupture and metabolic activity to begin.

MS media is a medium capable of inducing the growth of orchid seed protocol *V. helvola*, while VW and organic media are not yet capable. MS media is richer in nutrients than VW media. Based on the research results of Istiqomah [3], the growth of Phalaenopsis amabilis L. Blume plantlets was better on MS media than on VW media. According to Latifah et al. [16], MS media has the right content for plant needs to encourage rapid plant growth and development.

Coconut water contains cytokinins, auxin and several important substances for culture growth, namely amino acids, nucleic acids, purines, organic acids, sugars, vitamins and minerals [17]. The banana extract contains cytokinins, iron, potassium and vitamin B1, which can stabilize the pH of the media [18-19]. Potato extract contains carbohydrates, amino acids, vitamins, potassium, iron, magnesium, and vitamins B1, B6 and C [20]. The contents of organic compounds added to VW media and organic media could not induce the formation of *V. helvola* orchid seed protocols. This research shows that different media compositions will provide different growth responses. From the observations, MS media added with tomato juice is the right medium for the growth of *V. helvola* orchid seeds. According to Dwiyani [11], *Vanda tricolor* orchid germination media requires tomato extract.

Conclusion

Vanda helvola orchid seeds, in MS media, the seed changes reach stage 6 while VW (Vaccine and Went) media and organic media respectively reach stages 4 and 2. MS (Murashinge and Skoog) and VW (Vaccine and Went)

media can induce germination of orchid seeds while the media The best method for forming protocols is MS media.

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