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In Vitro Culture of Orchids Grammatophyllum scriptum lindl. from Ambonese banana Peel in Foliar Fertilizer Medium

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ABSTRACT

The sustainability of Grammatophyllum scriptum orchids in nature is threatened due to excessive harvesting which results in changes and damage to the orchid's growing habitat. G. scriptum orchid is one of the endemic orchids that has uniqueness and high economic value. In vitro culture propagation of G. scriptum orchids is carried out to multiply and maintain the existence of the orchid. The use of alternative media in vitro culture is carried out to minimize the cost of making the medium and utilize materials available in nature. The utilization of Ambonese banana peel waste that has not been carried out optimally is considered to be one of the choices as an alternative medium in propagation of G. scriptum orchids This study aims to determine the most appropriate combination of foliar fertilizer and banana peel concentration for in vitro multiplication of orchid explants and to examine the effect of the combination of leaf fertilizer and banana peel concentration on the multiplication of orchid explantsVitro. The study will be carried out using a single-factor experimental method with 8 treatments, arranged according to the Complete Randomized Design (RAL). The treatment tried was in vitro culture of G. scriptum orchids on Growmore media and Ambonese banana peels including inner banana peels of 50g/L and 100g/L; outer banana peel 50 g/L and 100 g/L; banana peel combined 50 g / L and 100 g / L. The results showed the addition of Ambon banana peel had an influence on the percentage of life and growth of shoots, leaves and roots of G. scriptum orchid plants Treatment with the addition of a combined banana peel of 50 g / L in leaf fertilizer medium provides the best growth in terms of the percentage of life, plant height growth, leaves and plant roots.

Keywords: *Tissue culture, medium substitution, Grammatophyllum orchid, banana husk*

INTRODUCTION

Orchid Grammatophyllum scriptum lindl. is one of the orchids that are in great demand for cultivation because of its beautiful and large flowers. The uniqueness of this type of orchid lies in the shape and color of the flowers, which are yellowish-green with brown spots that resemble tiger patterns with flower widths up to 4.5 cm and flower stalks with a length of up to 1 meter. Another uniqueness possessed by this orchid is that it has wind roots that emerge from the sidelines of the media that towers up, these roots function to absorb oxygen and also nutrients available in the

air. [1].

The existence of this orchid in nature becomes threatened due to excessive taking. Factors such as changes or destruction of growing habitats due to logging and land conversion are threats to the sustainability of natural orchids, and can lead to extinction if not balanced with conservation efforts. The threat that occurs in orchids in general is caused by human activities, namely the change or destruction of orchid growing habitat, either total damage, conversion into settlements, habitat fragmentation, taking from nature for trade or for other uses.a [2]. Preservation to maintain the existence of *G. Scriptum* orchids can be done propagation efforts through in vitro culture. Through this in vitro culture, orchid seeds that do not have endosperm can be grown on artificial media and develop into new individuals. In nature, of the millions of orchid seeds, only 5% are able to grow, while in vitro culture up to 90% of seeds can germinate and grow [2].

In vitro culture propagation, a medium is needed that contains all macro and micro nutrients, vitamins and growth regulators needed by plants. In general, the medium used in vitro culture is MS (Murashige and Skoog) medium. The use of MS medium on a large scale can increase production costs, so efforts need to be made to substitute media using leaf fertilizer medium which is easier to obtain and can save production costs. The concentration of foliar fertilizer that can be used in in vitro culture ranges from 3-4 g / l [3][4].

The use of organic matter in vitro culture medium has been widely done, including: coconut water, bananas, bean sprouts, tomatoes, avocados, and others. Coconut water contains organic acids, sugars, amino acids, vitamins, and growth regulators such as cytokinins, auxins, and gibberellins that can promote explant growth. Other organic materials also contain various nutrients and minerals needed for explant growth [3][4].

The use of waste, especially banana waste in the form of banana peels, has not been carried out in the invitro culture of G. Scriptum orchids. Banana peels contain fiber, calcium, phosphorus, sugar, protein, fat, carotene, and anthocyanins [5]. It also contains iron, B vitamins, vitamin C, water, pectin, and lignin [6]. Problems that arise in the use of banana peels include: the outer banana peel contains pectin and lignin which are high enough, so that if used in vitro culture, it is feared that it can inhibit the growth of explants. In addition, it is not yet known how much banana peel waste concentration is right for the growth of G. scriptum explants. Therefore, in this study, the use of banana waste is divided into 3 parts, namely the outer skin, inner skin, and combined skin (whole). The purpose of this study was to examine the effect of using banana peels, and determine the most appropriate banana peel concentration for the growth of *G. Scriptum* explants

MATERIALS AND METHODS

The ingredients used include: *G. scriptum* orchid explants in the form of young shoots from seed germination in vitro aged 4 months, MS medium, leaf fertilizer medium (growmore), agar-agar, sugar, coconut water, ambon banana peel, BAP (*Benzyl amino purine*), NAA (*Nafthalene Acetic Acid*), alcohol, betadine, spiritus. The tools used: LAF (*Laminar Air flow*), petridish, dissecting kits (tweezers, scalpel, scissors), bunsen, handsprayer, autoclave, glassware, pH meter, analytical scale, and culture bottle.

The study was conducted using a single-factor experimental method with 8 treatments, which was compiled using a Complete Randomized Design (RAL). Each treatment uses 3 repetitions and each test has 3 samples. The treatment tried, namely:

A. MS + BAP 2 ppm + NAA 0,5 ppm
B. PD 3 g/l + AK 150 ml/l
C. PD 3 g/l + AK 150 ml/l + KD 50 g/l
D. PD 3 g/l + AK 150 ml/l + KD 100 g/l
E. PD 3 g/l + AK 150 ml/l + KL 50 g/l
F. PD 3 g/l + AK 150 ml/l + KL 100 g/l
G. PD 3 g/l + AK 150 ml/l + KG 50 g/l
H. PD 3 g/l + AK 150 ml/l + KG 100 g/l

Description:

MS: Murashige-Skoog, PD: Foliar Fertilizer, AK: Coconut Water, KD: Inner banana peel, KL: Outer banana peel, KG: combined/whole banana peel

Sterilization Tools

The tools used are sterilized using an autoclave. After the tool is washed clean, glassware tools and dissecting kits are wrapped in umbrella paper then sterilized using an autoclave for 1 hour with a pressure of 1 atm at 120 ° C. Dissecting kits when they will be used for inoculation, are sterilized by burning sterilization, namely by soaking in alcohol and then burning on a bunsen lamp until the alcohol is dry.

Making Planting Media

Sterilization of banana peels is carried out based on research from Rahayu & Prayogi (2014), namely banana peels are washed with detergent, soaked in bactericides and fungicides for 12 hours, then boiled, and mashed with a blender. The banana peel is further separated according to the treatment. The inner banana peel is scraped off using a spoon, this part is the inner banana peel, while the rest is the outer banana peel. While the combined banana peel is the banana peel as a whole. While coconut water sterilization is done by filtering using filter paper and added in a medium mixture, then the medium is sterilized using an autoclave for 30 minutes with a pressure of 1 atm and a temperature of 120 ° C [8].

The control medium used is MS medium which is made by mixing a solution of macro, micro, vitamin MS, and mio inositol [9]. In addition, BAP 2 ppm, and NAA 0.5 ppm, sugar 30 g / l. Media Foliar fertilizer is made by mixing leaf fertilizer 3 g / l with sugar 3 g / l, coconut water 150 ml / l and banana peel 50 g / l and 100 g / l according to treatment. All medium solutions are added agar 7 g/l and cooked until they dissolve. The medium solution is divided into 20 ml of each culture bottle according to the treatment then covered with plastic, tied with rubber, and sterilized using an autoclave with a pressure of 1 atm at 120 ° C for 30 minutes.

Inoculation and Incubation of Explants

Inoculation of G. Scriptum explants is performed inside the LAF. The explant is taken aseptically using sterile tweezers and placed in a petridish, then cut approximately 1 cm long. The explant pieces are further inoculated into the treatment medium.

All inoculated explants are placed on a culture rack, in an incubation chamber with a temperature setting of 26°C with a lamp light of 1000-4000 lux.

RESULTS AND DISCUSSION

In vitro Culture Success

The main factors that determine success in in vitro culture are the percentage of live explants, the percentage of browning explants, and the percentage of contamination explants. The G. *scriptum* orchid explant used is the result of aseptic germination of orchid seeds. Data on the success of in vito culture of *G. scriptum* orchids are listed in Table 1.

Tabel 1. Success of In Vitro Culture of Orchids G. Scriptum on various mediums for 8 weeks

Treatment	Percentage (%)		
	Live	**Contamination**	***Browning***
MS+BAP 2 ppm +NAA 0,5 ppm	100	0	0
PD 3g/l + AK 150 ml/l	77,78	0	22,22
PD 3 g/l + AK 150 ml/l + KG 50 g/l	100	0	0
PD 3 g/l + AK 150 ml/l + KG 100 g/l	100	0	0
PD 3 g/l + AK 150 ml/l + KL 50 g/l	100	0	0
PD 3 g/l + AK 150 ml/l + KL 100 g/l	44,44	55,56	0
PD 3 g/l + AK 150 ml/l + KD 50 g/l	44,44	55,56	0

PD 3 g/l + AK 150 ml/l + KD 100 g/l	77,78	22,22	0
Rata-rata	80,56	16,67	2,78

Description: MS = Medium Murashige &; Skoog, PD = Foliar Fertilizer, AK = Coconut Water, KG = Combined Banana Peel, KL = Outer Banana Peel , KD = Inner Banana Peel

Based on Tabel 1, the percentage of live explants as much as 80.56% was observed at week 0 to week 8 after inoculation. The percentage of live explants is observed based on the number of explants that do not experience contamination or *browning* in plant tissue or medium. A live percentage of more than 50% indicates success in vitro culture. Average explantcontamination in *orchid G. scriptum* by 16.67%. Contamination can occur in media or explants used in *vitro* culture. In this study contamination is characterized by the growth of white hyphae on the surface of the media surrounding the explant. Contamination of explants can be caused by several faktor, namely: explants that have been infected by microorganisms, the process during inoculation, or less sterile media [10]. According to Sunarjono (2002), environmental factors are also very decisive for the occurrence of contamination, where a room that is already very sterile can become unsterile in the rainy season, so that it can bring in bacteria and fungi from outside and increased humidity in the room can accelerate the development of microorganisms. Browning explants or explants that turn brownish have an average of 2.78%. An explant is said to be browning if more than 50% of the explant is browned. The browning process can occur allegedly due to cutting activities against the explants used so that there is an interaction between the *Polyphenol oxidase* (PPO) enzyme and the polyphenol content in the explant or can be caused by the sterilization process of dissecting kits during inoculation.

Growth of shoots

The explant used in the propagation of orchid plants is a young shoot of orchids Grammatophyllum scriptum lindl. The quality of plants produced in the in vitro culture process is greatly influenced by the explants used so that the use of young shoots is expected to provide good growth and results. Growth of shoots is observed in the 1st week after planting until the 8th week after planting. Data on shoot growth are in Table 2.

Tabel 2. The Effect of Adding Ambon Banana Peel on the Medium of Foliar Fertilizer and Coconut Water on Plant Height, When Shoots Appear and the Number of Orchid Shoots in the 8th Week After Planting.

Treatment	When buds appear (day -)	Number of buds	Plant Height (cm)	Number of leaves
MS+BAP 2 ppm +NAA 0,5 ppm	$20,44^{abc}$	$2,19^{a}$	$0,85^{c}$	$1,11^{bcd}$
PD 3g/l + AK 150 ml/l	$26,67^{c}$	$1,46^{bcd}$	$0,85^{c}$	$0,00^{d}$
PD 3 g/l + AK 150 ml/l + KG 50 g/l	$23,67^{bc}$	$1,67^{abcd}$	$1,01^{bc}$	$2,44^{a}$
PD 3 g/l + AK 150 ml/l + KG 100 g/l	$23,00^{bc}$	$2,04^{ab}$	$1,02^{bc}$	$2,33^{ab}$
PD 3 g/l + AK 150 ml/l + KL 50 g/l	$23,00^{bc}$	$1,88^{abc}$	$0,98^{bc}$	$2,00^{ab}$
PD 3 g/l + AK 150 ml/l + KL 100 g/l	$17,33^{ab}$	$1,24^{cd}$	$1,09^{abc}$	$1,55^{abc}$
PD 3 g/l + AK 150 ml/l + KD 50 g/l	$12,33^{a}$	$1,03^{d}$	$1,13^{ab}$	$1,33^{abc}$
PD 3 g/l + AK 150 ml/l + KD 100 g/l	$20,11^{abc}$	$1,41^{bcd}$	$1,31^{a}$	$0,67^{cd}$

Description: MS=Medium Murashige &; Skoog, PD=Foliar Fertilizer, AK=Coconut Water,
KG = Combined Banana Peel, KL = Outer Banana Peel, KD = Inner Banana Peel
Numbers followed by the same letter showed no real difference on the DMRT test.

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Based on the results of the fingerprint notation in Table 2., it can be seen that when the fastest shoots appear indicated by the medium of Foliar Fertilizer + Coconut water + banana peel in 50 g / l, while the longest emergence of shoots is the treatment of leaf fertilizer + coconut water. In Ambon banana peel and coconut water, there are cytokinins that can provide a growth response with a faster bud emergence time, while slow shoot growth tends to be caused by interactions between growth regulators produced by cells endogenously and exogenous growth regulators. This is in accordance with Gunawan's (1988) statement that the use of auxins and cytokinins exogenously can change the level of endogenous growth regulators produced by plant cells.

In the data on the number of shoots, the most shoots were in the medium MS + ZPT, which was not significantly different from the medium of leaf fertilizer + coconut water + banana peel combined 50 and 100 g / l, and the outer banana peel 50 g / l. There are plant height data, the highest shoots are found in the medium of leaf fertilizer + coconut water + banana peel in 100 g / l, which is not significantly different from banana peel in 50 g / l, and outer banana peel 100 g / l. This shows that the nutrient content and zpt in the medium of leaf fertilizer + coconut water + banana peel are almost the same as those found in the medium MS + zpt, so that it can produce the same number of shoots, and can be used as an alternative medium to replace MS.

Based on the results of fingerprints on the number of leaves, it can be seen that the number of leaves in the alternative medium, namely leaf fertilizer medium + coconut water + banana peel, almost all of them show better results than the number of leaves in MS medium, except in the medium with the addition of banana peel in 100 g / l. In addition, the best results were obtained from explants grown on leaf fertilizer medium + coconut water + banana peel combined 50 g / l. This is likely due to the content of nutrients, vitamins, and growth regulators in alternative mediums can stimulate better leaf turning. This is as revealed by Kristina & Syahid (2020), that coconut water contains nutrients N, P, K, Mg, Fe, Na, Mn, Zn, Ca, Vitamins (C, B5, inositol, pyridoxine, thiamin, ribofalvin), and zpt (kinetin, zeatin, and IAA). While the nutritional content of banana peels include: protein, P, K, Ca, Mg, S, and is thought to also contain several vitamins (Nasution et al., 2014; Aryani et al., 2018). Foliar fertilizer itself is very rich in macro and micronutrients, namely: N, P, K, Ca, Mg, S, Fe, Co, Cu, Mn, Zn, Mo, and B (Indary et al., 2023; Sembiring &; Maghfoer, 2018). The complete content of virgin elements in the alternative medium is thought to be a trigger for the growth of G. scriptum orchid explants which tend to be better than growth in MS medium.

CONCLUSION

The addition of combined banana peel with a concentration of 50 g / L in leaf fertilizer medium is the most appropriate concentration in vitro propagation of G. scriptum orchid culture by producing growth in the number of shoots, plant height, number of leaves and when better roots appear.

The use of leaf fertilizer medium with the addition of coconut water and banana peel can be used as an alternative medium for MS medium substitution in vitro culture of G. scriptum orchid plants.

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