

Substitution VW Medium Using Palm Date Puree in The Tissue Culture of *Grammatophyllum Scriptum*

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Abstract—The research aimed to determine date palm pure concentration and its effect on Tissue Culture of *Grammatophyllum scriptum*. The research used an experimental method with single factor and seven treatments arranged in a Completely Randomized Design. The treatments were: (1) Vacin & Went (VW) medium; (2) 50g/L date palm puree; (3) 100g/L date palm puree ; (4) 150g/L date palm puree; (5) 50g/L date palm puree + 30g/L sugar cane; (6) 100g/L date palm puree + 30g/L sugar cane; (7) 150g/date palm puree L + 30g/L sugar cane. All date palm puree treatments were combined with 3g/L foliar fertilizer. The observed variables were plant heights, number of leaves, number of shoots, the percentage of contamination, browning, life, and rooted explants. The result showed the growth and living performance of *G. scriptum* in the medium of 50g/L dates palm puree with and without sugar was not significantly different on the VW medium. It indicated that the growth of *G. scriptum* in this combination of such media was relatively similar with those grown on VW medium. The medium of 50g/L dates palm puree combined with 3g/L foliar fertilizer could substitute the VW medium in the subculture of *G. scriptum*.

Keywords—*in vitro culture, orchid, plant puree, subculture*

I. INTRODUCTION

Grammatophyllum scriptum is the biggest orchid in the world. It is native to Southeast Asia and is found in low-lying coastal areas. Orchid multiplication by tissue culture is one of the efforts to preserve the diversity of orchids. Conventional orchid propagation is difficult to do as the seeds of orchid are very small, and smooth and have no endosperm [1]. In vitro, the culture medium of orchids contains various kinds of macro and micro nutrient elements, vitamins, and plant growth regulator to support plant growth. Vacin and Went (VW) medium is the most widely used one for the in vitro culture of orchid. However, when used on a large scale, it has led to inefficiency for the high price. There, in response, has been some attempts to substitute the media using eco-friendly natural ingredients with low price. The synthetic hormones as auxin and cytokines are substituted using natural hormones in plant extract (coconut water, tomatoes pure, banana pure, and potatoes pure). Foliar fertilizers contain macro and micro nutrient elements for plant growth, and it can substitute VW medium. 3 gr/L foliar fertilizer in the medium of in vitro culture could germinate the seed of *Dendrobium*, *Vanda*, and *Cattleya* could germinate on in vitro medium with 3 gr/L foliar fertilizer.

Natural ingredients are the source of sugar, vitamins, growth regulatory substances, and amino acid. One of the natural ingredients containing vitamins, minerals, and sugar sources and potentially to be used as a substitution in the in vitro culture medium is dates palm. Most of the palm fruits contain sugar glucose, fructose, and sucrose. 60% carbohydrate is on Ruthab date (wet date palm) and 70% is dried dates. The date also contains 20% protein, 3% fat, and the rest IE substance mineral salts and iron. 100 gr dried date contains 90 IU vitamin A, 93 mg thiamine, 114 mg riboflavin, 2 mg niacin and 667 mg potassium. Other elements of date palm are 1.2 mg iron, 667 mg potassium and 63 mg phosphorus [2]

The use of organic materials and plant extracts in tissue culture has been widely studied, including the use of coconut water in *Pogostemon sp.* [3], *Curcuma* [4], Alfalfa [5] ; soybean and corn extract on phalaenopsis orchid seed germination [6], and banana extract on tissue culture of phalaenopsis [7]. The use of palm dates purees as organic material in the tissue culture medium, nevertheless, has not been done yet. This research aimed to examine the influence of pureed dates as an alternative media on the subculture of *G. Scriptum* and to determine the most appropriate concentration of date palm puree for tissue culture of *G. scriptum*

II. METHODS

Some fourth-day seedling of *G. scriptum* were used as explants for subculture. Vacin and Went medium was used as a control medium. The treatment medium for substitution consisted of 50 g/L, 100 g/L, and 150 g/L date palm puree combined with foliar fertilizer (3 g/L Hyponex [8]) with or without 30 g/L sugar.

The research was conducted at Tissue Culture Laboratory, Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta, from November until January. This research method used some laboratory experiments with 8 treatments and was arranged in a Complete Random Design (CRD) with three replications and 3 samples. The treatments included: (1). Vacin and Went medium (VW); (2) 50 g/L dates palm puree; (3) 100 g/L dates palm puree; (4) 150 g/L dates palm puree; (5) 50 g/L dates palm puree + 30 g/L sugar; (6) 100 g/L dates palm puree + 30 g/L sugar; and (7) 150 g/L dates palm puree + 30 g/L sugar. All dates palm puree treatments were combined with 3g/L foliar fertilization. VW medium

composition was based on Vacin and Went medium [9] and [10].

The observed data included the percentage of contamination, percentage of browning, percentage of life, and percentage of rooted explants, number of leaves, number of shoots, and plant height. The data were analyzed by means of Anova (Analysis of variance) at 5% error level. If there were significant differences, the data we analyzed used the Duncan Multiple Range Test (DMRT) at 5% error level.

III. RESULTS AND DISCUSSION

We have grown *G. scriptum* by tissue culture using an alternative medium to substitute VW medium. The data in Table 1 indicated that in plant height, the treatment of 50 g/L date palm puree either with or without sugar showed no significant difference compared to the medium VW as a control. Medium foliar fertilizer added with coconut water and date palm puree contained macro-micro nutrients, vitamins and plant growth regulator needed for plant growth. Hyponex foliar fertilizer consisted of macro and micro elements, i.e., N, P, K, Ca, Mg, S, Fe, Co, Mn, and Zn (Nishimura, 1982) and [8]. Coconut water was composed of N, P, K, Mg, Mn, Fe, vitamin, auxin, cytokine, and gibberellin. Meanwhile, the components of date palm puree included macro-micro nutrients, vitamin, thiamin, riboflavin, glucose, fructose, biotin, folic acid, and ascorbate acid [11], [12].

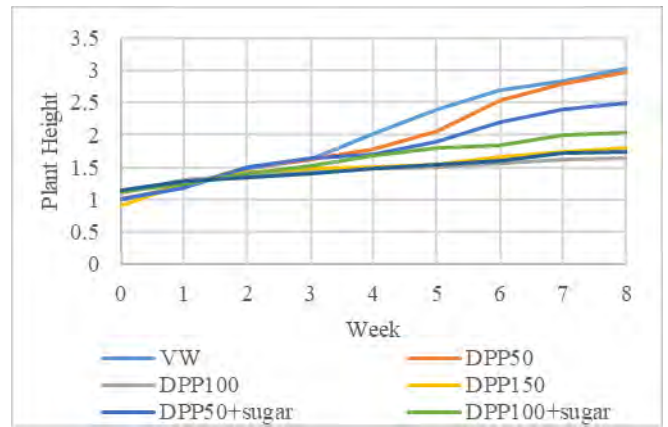
The number of leaves and the number of shoots of *G. scriptum* in the foliar fertilizer medium + dates palm sugar with/without sugar were fewer than that of VW medium. VW medium consisted of synthetic macro-micro nutrients in a simple form and easily to be absorbed by explants, while the nutrients in the substitution medium were available in the complicated compositions and were relatively difficult to be absorbed by explants. Data in Table 1 indicated that palm dates puree combined with foliar fertilizer and coconut water as medium substitutions were not quite good for multiplication, but it could substitute VW medium for subculture or enlargement seedling.

Table 1. The average height, number of leaves, and the number of shoots Orchid *Grammatophyllum scriptum* by tissue culture for 8 weeks

Treatment	Plant height (cm)	The Number of Leaves	The Number of Shoots
VW	3.02 a	5.44 a	3.11 a
50g/L DPP	2.98 a	4.11 b	1.11 b
100g/L DPP	1.64 c	3.11 bc	1.11 b
150g/L DPP	1.81 bc	2.33 c	1.00 b
50g/L DPP + 30g/L sugar	2.50 ab	3.33 bc	1.00 b
100g/L DPP + 30g/L sugar			
sugar	2.04 bc	3.44 bc	1.00 b
150g/L DPP +30g/L sugar	1.74 bc	3.22 bc	1.00 b

The average following a similar letter in one column showed no significant difference in accordance with DMRT at 5% level α error.

VW: Vacin and Went medium; 50-150g/L DPP: 50-150g/L dates palm puree



As seen in Figure 1, there were some variations of *G. scriptum* plant growth in the different medium, but there was one similarity in all treatments in which the growth of seedlings increased every week. It also showed that VW medium and 50g/L dates palm puree without sugar provided the best growth of *G. scriptum* in the end of observation (8 weeks). It indicated that 50 g/L dates palm puree could substitute VW medium in the plant growth of *G. scriptum* by tissue culture. The graph shows the plant growth of *G. scriptum* for eight weeks with the different medium. *DPP = 50-150g/L Dates Palm Puree; 30g/L sugar

Table 2. The percentage of contamination, browning, life, and rooted of *G. scriptum* by tissue culture within 8 weeks

Treatment	Contamination (%)	Browning (%)	Life (%)	Rooted (%)
VW	0	0.00	100.00	100.00
50g/L DPP	0	0.00	100.00	100.00
100g/L DPP	0	33.33	66.67	55.56
150g/L DPP	0	66.67	33.33	11.11
50g/L DPP + 30g/L sugar	0	0.00	100.00	100.00
100g/L DPP + 30g/L sugar	0			
sugar		44.44	55.56	33.33
150g/L DPP + 30g/L sugar	0			
sugar		100.00	0.00	0.00

The average following a similar letter in one column showed no significant difference according to DMRT at 5% level α error.

VW: Vacin and Went medium; 50-150g/L DPP: 50-150g/L dates palm puree

Table 2 shows that the explants in all the treatments were not contaminated. This research used explants from previous tissues culture research; however, to eliminate the contaminants, explants were firstly sterilized using 5% Clorox for 5 minutes before inoculation. Previous researches mentioned that explants sterilization using 5% Clorox for 3-5 minutes was effective for suppressing the contaminations on the tissue culture of *Pogostemon sp.* [13] and [14]; *Zea mays* [15]; and sugar cane [16]. Table 2 also mentions that there was no explant browning on medium and medium VW with 50 g/L dates palm puree with and without sugar. Meanwhile, in the medium of 150 g/L dates palm puree with 30 g/L sugar, entirely explant experience browning (100%). The medium with 150 g/L dates of palm puree with 30 g/L sugar had a very

high sugar content in the medium that stimulated the maillard reactions, a chemical reaction between amino acids and reducing sugars making browned on plant tissue [17] and [18].

The percentage of life explants represented the number of explants that could grow on tissue culture medium without browning or contamination. The highest life explant percentage could be seen on the VW medium and medium of 50 g/L dates palm puree either with or without sugar (100%). The ability explants to grow in that medium indicated that explants could adapt with the lower sugar concentration, and could absorb nutrients from the medium. The percentage of rooted explants on the VW medium was 100% equal to the medium of 50 g/L dates palm puree with or without 30 g/L sugar. It indicated that those media provided the favorable condition for growing explants. Adventitious roots began to form in the stem of most species after cutting if the medium condition was appropriate [19]. The concentrations of sugar in the VW medium and 50 g/L dates palm puree with and without sugar were not as much as in other treatments so that explants could easily absorb the nutrients from those media. These nutrients were used to activate the enzyme to produce an indigenous plant growth regulator, which is important for the root growth. The endogenous plant growth regulator especially cytokine was involved in the regulation of adventitious root formation [19]

IV. CONCLUSION

Dates palm puree medium combined with foliar fertilizer could be used for the growth of *Grammatophyllum scriptum* by tissue culture. The growth and living performance of *G. scriptum* in the medium of 50 g/L dates palm puree with and without sugar was not significantly different on the VW medium. It indicated that the growth of *G. scriptum* on this combination of such medium was relatively similar with those grown in VW medium. 50g/L dates palm puree combined with 3g/L foliar fertilizer could substitute the VW medium in the subculture of *G. scriptum*.

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