BioEksakta: Jurnal Ilmiah Biologi Unsoed
Volume 3 Nomor 4 (2021): 190-195
E-ISSN: 2714-8564

The Effect of Date Palm Extract *(Phoenix dactylifera* L.*)* on Orchid Plantlets *(Vanda tricolor* Lind.*)* Growth Using *In Vitro* Culture

Resita Nursechan*, Kamsinah, Iman Budisantoso

Fakultas Biologi, Universitas Jenderal Soedirman, Purwokerto 53122, Indonesia
Email: resita.nursechan@mhs.unsoed.ac.id

Rekam Jejak Artikel:

Diterima : 19/05/2021
Disetujui : 10/04/2022

Abstract
Vanda tricolor Lindl. is widespread in Java, Bali, and Sulawesi, *V. tricolor* on natural habitat became scarce due to forest destruction and natural disasters and human activities. To prevent the extinction it can be done by the propagation of orchids using in vitro culture. The addition of dates extract (*Phoenix dactylifera* L.) containing carbohydrates to increase growth and differentiation of cells in orchids. This study aimed to determine the effect of date palm extract on the growth of *V. tricolor* L. plants and determine the best dose of date palm extract on the growth of *V. tricolor* plantlets. This research was conducted experimentally with a Completely Randomized Design (CRD). The addition of dates on Vaccint & Went / VW media consisting of 6 treatments, that are : 0 g/L, 50 g/L, 100 g/L, 150 g/L, 200 g/L, 250 g/L, each treatment repeated 3 times so there are 18 trial units. The source of the explants used was orchid plantlet (*V. tricolor* L.). The parameters include the number of roots, root length, number of leaves, plantlet height, and leaf length. The research data has been analyzed using Analysis of variance (ANOVA) with a confidence level of 95% and 99%. The result of this research showed that the addition of date palm extract give a result not significant for the number of leaves, the number of roots, the longest leaf length, the longest root length, the plantlet height of the *vanda tricolor* orchid in vitro
Key words: *Phoenix dactylifera L, Vanda tricolor Lind., in vitro*

INTRODUCTION

Orchid plants belong to the family Orchidaceae which is one of the largest flower plant families. *Vanda tricolor* L. is a type of natural Vanda orchid that has a high economic value. This type of orchid can be used as a cross parent to produce new Vanda flowers with unique varieties. Orchid *V. tricolor* L. has many advantages, such as the size of the flower is quite large, there are variations in terms of shape, color, and flower pattern. Flowers *V. tricolor* L. are generally white ivory patterned yellow, whitish-pink, purplish-red, or red with brown spots (Adiguna *et al*., 2018).

According to Dwiyani *et al.* (2012), Species *V. tricolor* L., in its original habitat, began to be scarce due to forest damage, natural disasters, and human actions. It is necessary to conduct an effort both in situ and ex situ conservation. Ex-situ conservation can be done by propagating plants outside their habitat or planted in the yard, propagating in an orchid nursery or in the experimental garden of research institutes and universities. Propagation by in-vitro culture is a beneficial method for endangered species for conservation purposes and an advantageous method for orchids. Because orchid seeds do not have food reserves, so in nature they symbiosis with certain fungi to germinate with very slow growth (Dwiyani *et al*., 2012).

Culture in vitro is the cultivation of plants in an aseptic condition by using a sterile medium in a culture bottle (Dodds *et al*., 1985). This method is considered effective because the results can be obtained in large numbers and in a short time. One of the stages of in vitro culture is a subculture. Subculture is one of the steps that need to be done to ensure plants get enough nutrients for optimal growth by transferring explants from the old culture media to new culture media and can form buds and roots with the appropriate combination and concentration of hormones (Febryanti *et al*., 2017).

Dates are a good food source with high nutritional value. Dates contain more than 3,000 calories/kg. Protein content in dates is 5.77%, fat by 10.62% and carbohydrates by 77.52% (Nehdi *et al*., 2010). Dates contain fruit components that are mostly reducing sugars, namely glucose and fructose (Retnowati & Kusnadi, 2013). According to Praristiya, (2019) the date extract contains tryptophan amino acid. Dates also contain several types of vitamins, such as nicotinic acid (niacin) of 2.2 mg, vitamin C (0.002-0.02 %), thiamin (0.09 mg), and riboflavin (0.10 mg) (Mahmoudi *et al*., 2008). The flesh of dates contains 60 -65% sugar,

about 2.5% fiber, 2% protein and less than 2% consists of fats, minerals and pectin elements (Swito & Randarini,2015).

Carbohydrates and high sugar levels are a source of energy in the metabolic process. Carbohydrates also have a very important role in stimulating plant growth (Sallolo *et al*., 2012). Sucrose is the ultimate product of photosynthesis (Lehninger, 1982). Protein plays a role in daily cell activities such as the process of division, replacing damaged or old cells (Parman, 2007). Minerals are needed for growth. If not fulfilled, then plants can not complete their life cycle. Pectin acts as an adhesive and maintains tissue and cell stability (Mahmoudi *et al*., 2008).

Based on the description above, the problem that can be examined in this study does date palm extract affects *V. tricolor* L. plantlets growth and which is the best concentration of date palm extract for the growth of *V. tricolor* L plantlets.

This research aims to determine the effect of date palm extract on the growth of *V. tricolor* L. plantlets in vitro culture and knowing the best dose of date palm extract on the growth of *V. Tricolor* L. plantlets culturally in vitro. This study's results are expected to provide scientific information about the effect of giving the best dose of date extract for the growth of *V. tricolor* L. plantlets in vitro culture.

MATERIAL AND METHOD

The material used in this study is the planlet orchid *V. tricolor* L. cultural results obtained from the Faculty of Biology Jenderal Sudirman University, media Vacin & Went (VW), agar, date extract, aquadest, NaOH 1 N, HCl 1 N, sugar, and alcohol 96%. Tools used are beaker glass, hot plate and magnetic stirrer, analytical scales, measuring cups, erlenmeyer, petri cups, drip pipettes, aluminum foil, micropipettes and tips, pH meters, spatulas, pans, duran bottles, culture bottles, pp (polypropylene) plastics, rubber bands, label papers, bunsens, filter paper, autoclaves, ovens, wrappers, scalpels, tweezers, Laminar Air Flow (LAF), refrigerators, sprayers, stoves, and rags.

This research was conducted in the Laboratory of Plant Physiology, Faculty of Biology, Jenderal Soedirman University Purwokerto for ± 3 months, from September to December 2020. The experimentally with a Complete Randomized Design (RAL). Consists of 6 treatments namely I_0 = 0 g/L, I_1 = 50 g/L, I_2 = 100 g/L, I_3 = 150 g/L, I_4 = 200 g/L, I_5 = 250 g/L. Each treatment is repeated 3 times, so that obtained 18 experimental units. The variables observed in this study are the independent variables and the dependent variable. The independent variable was date extract with various concentrations, while the dependent variable was plantlet growth. The parameters observed were the addition of the number of leaves, the length of the longest leaves, the addition of the number of roots, the length of the longest roots and the plantlets' height.

Extracts was done by weighing as much as 150 grams, then put into mortar and mashed it using pestle. The date palm extract that has been made will be form paste as much as 150 gr, each treatment obtained date palm extract as much I_0 = 0 g, I_1 = 10 g, I_2 = 20 g, I_3 = 30 g, I_4 = 40g, I_5 = 50 g.

The equipment to be used first washed with a detergent until it is clean, then dried. Non-glass tools such as tweezers are wrapped in paper, whereas glassware such as culture bottles are wrapped in aluminum foil, then sterilized using an autoclave at 121 ℃ and pressure of 0,15 MPa for 30 minutes. Then stored in the oven with a temperature of 70 ℃. Tools, such as tweezers, are sterilized again when they are used in LAF by burning the tip on the fire until the tip turns red (flaming), then cooled. The culture bottles which contained treatment media were sterilized using an autoclave at 121 ℃ and a pressure of 0.15 MPa for 20 minutes.

LAF was sterilized by using ultraviolet (UV) lamps for 60 minutes. Then LAF was sprayed with 96% alcohol. All tools and materials to be used are sprayed with 96% alcohol then put into LAF. The *V. tricolor* L. plantlet used for this study came from the Plant Physiology Laboratory collection, Jenderal Soedirman University. Planting is carried out in LAF under sterile conditions. The mouth of the bottle is preheated with bunsen fire to prevent contamination. Scalpel and tweezers are always heated before and after use to maintain sterilization from the device. Planting is done by taking a plantlet from a bottle with sterile tweezers and then placed in a petri dish that has been covered with filter paper. Healthy plantlets with similiar size have 2 leaves, 1 root, and planlet height more or less 5 mm has been selected, then planted by tweezers on VW media I_0 = 0 g/L planted with 3 planlets (*V. tricolor* L.), I_1 = 50 g/L planted with 3 plantlets (*V. tricolor* L.), I_2 = 100 g/L planted with 3 orchid plantlets (*V. tricolor* L.), I_3 = 150 g/L planted with 3 orchid plantlets (*V. tricolor* L .), I_4 = 200 g/L planted with 3 orchid plantlets (*V. tricolor* L.), I_5 = 250 g/L planted with 3 orchid plantlets (*V. tricolor* L.). The mouth of the culture bottle was heated, after that closed the culture bottle using pp plastic, then bound with a rubber band and covered with a wrapper. The bottles that have been filled with orchid plantlets are labeled according to the treatment and planting date, then placed on a culture rack.

The culture bottle was placed in the culture rack for 12 weeks. The culture chamber condition was maintained at a temperature of 18-22 ℃ with new intensity coming from TL lamps of 600-1000 lux, which is continuously lit. The culture bottles are kept clean to avoid contamination by spraying with 70% alcohol every 2 days.

Measurement of research parameters include:

1. Number of leaves

The increase of the number of leaves was observed by calculating how many leaves are

contained in the planlet. Data were collected at the end of the study 12 weeks after planting.

2. The longest leaf length (cm)

The longest leaf on a plantlet was measured in length using millimeter blocks. Data is collected at the end of the study (12 weeks after planting).

3. Number of roots

The number of roots was observed by counting how many roots are obtained on the plantlet. Data were collected at the end of the study (12 weeks after planting).

4. The longest root length (cm)

The longest root in a plantlet was measured by millimeter blocks to determine the root length. Data is collected at the end of the study (12 12 weeks after planting).

5. Plantlet height (cm)

The height of the plantlet was observed by calculating the height of the shoot on the plantlet. The measuring tool used is millimeter blocks. Data were collected at the end of the study (12 weeks after planting).

Data analysis

The research data was analyzed using Analysis of variance (ANOVA) with a confidence level of 95% and 99%. If it has a real effect, then it will be continued with the Least Significant Difference test (LSD) at a 95% confidence level.

RESULT AND DISCUSSION

The results observations of the growth *V. tricolor* L. planted in VW media visually shows the presence of growth characterized by addition of the number of roots, the number of leaves, the addition of root length, the addition of leaf length, as well as the addition of the number of shoots.

The analysis variance results obtained that the treatment of date extract does not have a real influence on the growth of the number planlet leaves *V. tricolor* L. This is because the compounds available in the media have been fulfilled, so the addition of date extract does not affect the growth of the number planlet leaves *V. tricolor* L., it is supported by Lestari & Deswiniyanti (2016) said that vacin and went media consists of macronutrients and micronutrients in the form of inorganic salts with the appropriate amount for plant growth, especially orchids, various compositions of growing media have been formulated to optimize the growth and development of cultured plants. In addition Dwiyani *et al*. (2016) said that the orchid *V. tricolor* L., has a long vegetative period, so a short observation time (12 weeks after planting) has not shown the results as expected.

The number of leaves is one of the indicators of plant growth and can be used as supporting data to explain the growth process. The leaves are the place where photosynthesis takes place, which is the formation of carbohydrates in the leaves. Leaf observation is needed as a growth indicator to explain the growth processes that have occurred, such as in the formation of plant biomass. The more leaves that appear on the explants, indicates better explant growth. The number of leaves in the plant plays a significant role, this is related to plants' ability to perform photosynthesis and various other metabolisms. A large number of leaves will produce a lot of photosynthates, so the growth of plants will be better (Tobing, 2019).

Based on the observation data the number of leaves (Table 1.), It can be seen each date palm extract treatment given resulted in a different average number of leaves, the highest number was produced in treatment I_1 (50 g/L) with an average of 6.33 leaves/explant. According to Mahmoudi *et al*. (2008) Dates contain several types of vitamins, such as nicotinic acid (niacin) of 2.2 mg, vitamin C (0.002-0.02%), thiamin (0.09 mg), and riboflavin (0.10 mg). The addition of vitamins to the culture media can stimulate the growth of orchid plant tissues and organs (Widiastoety *et al*., 2009). Vitamin B1 (thiamin) belongs to the phytohormone group, which is a substance that in small amounts can stimulate plant growth (Silviasari *et al*., 2014). Meanwhile, based on observational data (Table 2.) The least number of leaves was produced by plantlets treated with date palm extract I_4 (200 g/L) with an average of 3.67 leaves/explant. This is because the addition of 200 g/L of date palm extract can increase the concentration of sugar in the media, so that can inhibit the growth of plantlets, this is following the opinion of Silviasari *et al*. (2014) which states that high concentrations of sucrose in culture media can inhibit the growth of somatic cells. This shows that the treatment given responds to an increased number of leaves in plantlets. However, statistically it has not a real effect.

Table 1. Tabulation Data from Observation The Number of New Leaves at Various Doses of Date Palm Extract

Date	Repetition			Total	Average
	1	2	3		
I_0	5.0	4.5	3.5	13.0	4.333
I_1	5.5	5.0	8.5	19.0	6.333
I_2	2.0	5.0	4.5	11.5	3.833
I_3	5.5	5.0	5.0	15.5	5.167
I_4	3.0	3.5	4.5	11.0	3.667
I_5	5.0	4.0	4.5	13.5	4.500

Table 2. Tabulation Data from Observation of The Number of New Roots at Various Doses of Date Palm Extract

Date	Repetition			Total	Average
	1	2	3		
I_0	2.0	1.5	1.5	5.0	1.667
I_1	2.0	2.0	3.5	7.5	2.500
I_2	1.0	2.0	1.5	4.5	1.500
I_3	2.5	1.5	2.5	6.5	2.167
I_4	1.0	1.5	1.5	4.0	1.333
I_5	3.0	2.0	1.5	6.5	2.167

Based on the analysis variance results the number of roots, it showed that the treatment of date palm extract had no significant effect on the growth of the number roots *V. tricolor* L. plantlets . This is similar to the number of leaves parameter, the compounds available in the media have been fulfilled, so the addition of date palm extract has no significant effect on the growth of the number of roots *V. tricolor* L. plantlets, the growth of *V. tricolor* L. roots is also very slow according to the opinion of Lestari & Deswiniyanti (2016).

Based on the data observation the number of roots (Table 2.) shows that the dose of date extract produced an average number of different roots in each treatment the most amount produced in the treatment I_1 (50 g/L) with an average of 2.5 roots/explants. While the least number of roots produced by planlets given the treatment of date extract I_4 (200 g/L) with an average of 1.33 roots/explant. This is following the research Silviasari *et al*. (2014), in addition to being a source of energy, sugar is also a new cell-forming material that in high concentrations can inhibit rooting. The high amount of carbohydrates or sugars in date extract will inhibit the growth of planlet because high concentrations of sucrose in cultural media can inhibit the growth of somatic cells (Silviasari *et al*., 2014).

Based on the analysis variance results, the longest leaf length showed that similar to the parameters on the number of leaves and the number of roots, the addition of date palm extract had no significant effect on the growth of long leaves *V. tricolor* L. plantlet. The addition of date palm extract is expected to increase the longest leaf growth because the carbohydrate content in date palm extract serves as an energy source for leaf growth. Carbohydrates have a positive effect on orchids, as in research by Aktar *et al*. (2008) the results showed that 15-20 g/L sucrose treatment gave good results on the length, width and number of orchid plantlet leaves compared to controls. Dates extract is a source of carbohydrates and a high source of calories. Therefore, it is very well used to support the growth of planlets, but the use of carbohydrates in high concentrations can inhibit leaves' growth because it can cause osmotic pressure. Silviasari *et al*. (2014) stated that visually plants that are under pressure due to osmotic influence are in the form of inhibition of leaf growth.

Based on observation data of the longest leaf length (Table 3.) in this study each dose of date extract produced a different average length of the longest leaves, the most amount produced in the treatment I1 (50 g/L) average of 16.67 mm. In comparison, the least leaf length was produced by plantlets treated with date palm extract I4 (200 g/L) with an average of 7.5 mm. The treatment given responds at low doses to increase V. tricolor L. plantlets' longest leaf length, but statistically it has not had a significant effect.

Based on the analysis of variance, it was found that the date palm extract treatment did not have a significant effect on the root length growth of *V. tricolor* L. plantlet. This is similar to the previous parameters, the addition of date palm extract does not have a real effect because the dose given is too high, so the high dose does not have a positive impact but is also not lethal, as the opinion of silviasari *et al*. (2014).

The length of root is the result of cell extension at behind tip meristem. The length of root can indicate the growth regulator substances and the nutrients available in medium work optimally. Long roots show the extent of nutrient absorption by plants and can fulfill the nutritional needs of plants. Besides that, the longer the roots are, it is expected that the plantlets will be strong enough to be acclimatized (Silviasari *et al*., 2014).

Table 3. Tabulation Data from Observations of the Longest Leaf Length at Various Doses of Dates Extract

Date	Repetition			Total	Average
	1	2	3		
I_0	8.5	13.5	9.0	31.0	10.333
I_1	14.5	22.5	13.0	50.0	16.667
I_2	7.5	15.0	12.5	35.0	11.667
I_3	11.0	9.0	12.0	32.0	10.667
I_4	5.5	6.5	10.5	22.5	7.500
I_5	12	11.0	5.5	28.5	9.500

Table 4. Tabulation Data of the Longest Root Length Observation Results at Various Doses of Date Extract

Date	Repetition			Total	Average
	1	2	3		
I_0	6.5	7.0	5.5	19.0	6.333
I_1	7.5	15.5	18.0	41.0	13.667
I_2	6.5	12.0	8.5	27.0	9.000
I_3	5.5	5.5	11.5	22.5	7.500
I_4	6.0	5.0	7.0	18.0	6.000
I_5	5.0	10.0	6.5	21.5	7.167

Table 5. Tabulation Data from Observation of Plantlet Height at Various Doses of Date Extract

Date	Repetition			Total	Average
	1	2	3		
I_0	8.5	13.5	9	31	10.333
I_1	14.5	22.5	13	50	16.667
I_2	7.5	15	12.5	35	11.667
I_3	11	9	12	32	10.667
I_4	5.5	6.5	10.5	22.5	7.5
I_5	12	11	5.5	28.5	9.5

Based on observation data of the longest root length (Table 4.) it showed that each treatment of date extract given produces a different average of the longest root length, the most amount produced in the treatment I_1 (50 g/L) with an average of 13.67 mm. While the root's length is at least produced by planlets treated with date extract I_4 (200 g/L) with an average of 6 mm. This shows that the treatment given responds to increase the longest root length in *V. tricolor* L. plantlets. However, statistically it has not had a significant effect.

Date extract contains the amino acid tryptophan (Praristiya, 2019). The amino acid tryptophan can help plants form endogenous auxins, so the auxin concentration is higher. High auxin concentrations can inhibit plant cell division and regeneration (Silviasari *et al*., 2014). The longest root average was obtained from addition of date palm extract in treatment I_1 (50 g/L) with an average of 13.67 mm. The treatment I_4 (200 g/L) obtained the lowest result that was 6 mm, it showed that root growth was inhibited. This condition is thought because the IAA concentration too high for a particular plant type will encourage ethylene synthesis which inhibits root elongation. IAA was synthesized from the amino acid tryptophan contained in date extracts. The addition of amino acids to the media is thought to be able to increase the IAA concentration formed by plants (Silviasari *et al*., 2014).

Based on the analysis variance results of plantlet heights, it showed that the treatment of date palm extract had no significant effect on the height of *V. tricolor* L. plantlets. This was similar to the previous parameter that the compounds available in the media had been fulfilled so the addition of date palm extracts has no significant effect on the growth of the plantlet heights of *V. tricolor* L. (Lestari & Deswiniyanti, 2016).

Based on the plantlet height observation data (Table 10.), It showed that each addition of date palm extract has a different average plantlet height, the highest amount was produced in treatment I_1 (50 g/L) with an average of 16.67 mm. This shows that the treatment given responds to increase the height of *V. tricolor* L. plantlets. However, statistically it has not had a significant effect. Carbohydrates and high sugar levels in date palm extract are a source of energy in the metabolic process, so they have a significant role in stimulating plant growth (Sallolo *et al.*, 2012). Lestari & Deswiniyanti (2017) stated that the carbohydrate content in media was the main factor of the primordial development of shoots and roots. However, based on observational data on plantlet heights (Table5.). The lowest average result was the addition of date palm extract I_4 (200 g/L) with an average 7.5 mm. This could be related to the ability of plant tissues to absorb nutrients. The natural extracts given in large amounts make the nutrients in the medium have a high concentration and the presence of fat in dates causes protein synthesis to run slower because it cannot be directly absorbed by orchid plantlets (Handayani & Isnawan, 2014).

CONCLUSION AND SUGGESTION

Based on the results and discussion it can be concluded that date palm extract does not affect the growth of *V. tricolor* L. orchids in vitro culture and The suggestion for this research are to conduct more research on the effect of date palm extract (*P. dactylifera* L.) on orchid planlets *V. tricolor* L. growth using in vitro culture with lower doses variety to obtain the best dose.

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