Analysis of Total Carbohydrate and Chlorophyll Content of The Orchid Plantlet [*Phalaenopsis amabilis* (L.) Bl.] Resistant Fusarium Wilt Disease

Analysis of Total Carbohydrate and Chlorophyll Content of The Orchid Plantlet [*Phalaenopsis amabilis* (L.) Bl.] Resistant Fusarium Wilt Disease

E Nurcahyani[1,2*], Sholekhah[1],Sumardi[1], H I Qudus[3]

[1]Master of Biology Study Program, Faculty of Mathematics and Natural Sciences, University of Lampung, Lampung, Indonesia
[2]Applied Biology Study Program, Faculty of Mathematics and Natural Sciences, University of Lampung, Indonesia
[3]Master of Chemistry Study Program, Faculty of Mathematics and Natural Sciences, University of Lampung, Lampung, Indonesia

email: endang.nurcahyani@fmipa.unila.ac.id*

Abstract. The moon orchid [*Phalaenopsis amabilis* (L.) Bl.] is a popular orchid in the community, native orchid from Indonesia, and included in the list of endangered species. The pathogenic fungus that often attacks orchid leaves is *Fusarium oxysporum*, which causes fusarium wilt. Control of diseases that do not cause negative impacts can be done using superior cultivars resistant to *F. oxysporum* infection, through in vitro selection in the medium with the addition of fusaric acid. Fusaric acid is a metabolite produced by several fungal species of the genus fusarium and at non-toxic concentrations it helps to induce phytoalexin synthesis, increase chlorophyll and carbohydrate content when pathogen infasion. The purpose of this study was to find out the total chlorophyll content, chlorophyll a, chlorophyll b, and total dissolved carbohydrate content. This study used a Completely Randomized Design (CRD) with one factor, namely the concentration of fusaric acid divided into 5 levels, namely 0 ppm, 10 ppm, 20 ppm, 30 ppm, and 40 ppm with 5 replications each. The results showed an increase in the total chlorophyll content, chlorophyll a, chlorophyll b, and total dissolved carbohydrate content.

Keyword: fusaric acid, *Fusarium oxysporum*, induced resistance, in vitro, *Phalaenopsis amabilis*.

1. Introduction

Orchidaceae is a family of very large flowering plants, with at least 20,000 species and 735 genera scattered throughout the world, especially in the equatorial region. The most popular type of orchid on the market is *Phalaenopsis amabilis* or known as the moon orchid [1]. The moon orchid is one of Indonesia's national flowers established by Presidential Decree No. 4/1993, as Puspa Pesona, in addition to jasmine (*Jasminum sambac* L.) as the nation's puspa, and giant padma flowers (*Rafflesia arnoldii* R. Br.) as a rare puspa [2]. Promising economic value makes moon orchids much hunted in nature that threatens their sustainability, so that the conservation status of moon orchids based on IUCN is endangered [3].

The obstacle faced in the cultivation of orchid is a disruption in the form of a disease that can make plants damaged and die. Several Phalaenopsis fungal diseases have been reported in Taiwan, including diseases caused by *Fusarium oxysporum* (*Fo*), *F. solani*, and *F. proliferatum* [4]. *Fo* causes fusarium wilt which interferes with the growth of orchids [5]. In the United States, fusarium wilt can cause crop death and decrease production by more than 50% and control with fungicides has not been able to overcome the disease [6].

One way to control disease that is efficient, effective and safe to the environment is to use resistant varieties. The use of high yielding varieties that are resistant to *Fo* is one important alternative disease control and does not cause negative impacts [7,8,9,10,11,12]. Development of *Fo* resistant plantlet varieties can be carried out among others by the in vitro selection method which is culturing explants in the form of tissue or organs on a medium containing selective concentration of fusaric acid [7,8,9,12].

Fusaric acid (FA) is a metabolite produced by several fungal species of the genus fusarium. FA chemically called 5-n-butylpicolinic acid. This acid can be toxic (concentrations of more than 10^{-5} M), so that it inhibits growth and regeneration of the culture, but at non-toxic concentrations (below 10^{-6} M) it helps to induce phytoalexin synthesis, a form of plant response to inhibit pathogenic activity [13]. The in vitro selection approach is reported to have produced resistant varieties in vanilla plantlet [14], *Arabidopsis thaliana* [13], and *Dendrobium sonia* [15].

Plants are under exposure to many types of pathogens. During the ongoing invasion of the pathogen, the physiology and cellular metabolism of the host plant are disturbed. Photosynthesis changes are the most common response to pathogen attack. Chlorophyll is a pigment that plays an important role in the process of photosynthesis, consisting of chlorophyll a and chlorophyll b [16]. There are three main functions of chlorophyll, namely utilizing solar energy, triggering the fixation of CO_2 into carbohydrates and providing an energetic basis for the ecosystem as a whole. This study aims to determine the specific expression of *P. amabilis* plantlet which resistant on *Fo* including levels of chlorophyll a, chlorophyll b, total chlorophyll and total dissolved carbohydrate content.

2. Materials and Methods

The medium used is Vacin and Went (VW), the medium is sterilized for 15 minutes. The sterilized VW medium is then added fusaric acid (FA) with a concentration of 0 ppm (control), 10 ppm, 20 ppm, 30 ppm, and 40 ppm for selection of disease resistance. The material used in the analysis of chlorophyll and total dissolved carbohydrate content is *P. amabilis* plantlet leaves which have been induced by AF. Chlorophyll analysis on resistance to Fo using the Miazek method (2002) [17]. *P. amabilis* plantlet leaves which were identical to 0.1 g were crushed with mortar (pestle), then added 10 mL 80% acetone. Then, the solution was filtered with Whatmann No.1 paper, and put in a flakon and then tightly closed. Sample solution and standard solution (80% acetone) were taken as much as 1 mL, and put in a cuvette. Absorption readings with UV spectrophotometer at wavelengths (λ) 649 nm and 665 nm.

Analysis of total dissolved carbohydrate content using the phenol-sulfur method [18]. The leaves of P. amabilis plantlets were taken and then weighed as much as 0.1 gram from each plantlet. The leaves were crushed with a mortar and given 10 ml of distilled water, then filtered with Whatman paper. Take 1 ml of the filtrate and add 1 ml of H_2SO_4 then add 2 ml of phenol. Furthermore, the filtrate is inserted into the cuvette and is read at a wavelength of 490 nm. The total dissolved carbohydrate content is calculated by making a standard solution of glucose.

3.Result and Discussion

The effect of giving FA as an inducer on *P. amabilis* plantlet can be known through the chlorophyll and total dissolved carbohydrate content of the plantlet. Plantlet was observed by comparing the plantlet without FA and the plantlet which was induced using FA with concentrations of 10 ppm, 20 ppm, 30 ppm, and 40 ppm. The results of the analysis of *P. amabilis* plantlet known to be an increase in the content of chlorophyll a, chlorophyll b, total chlorophyll and total dissolved carbohydrate content. The increased chlorophyll and total dissolved carbohydrate content of *P. amabilis* plantlet occurs along with the increased concentration of FA given. The results of the analysis showed that the mean comparison of total chlorophyll a, chlorophyll b, and total chlorophyll content between controls with the four concentrations of FA was significantly different. The results of the analysis of the content of chlorophyll a, chlorophyll b, and total chlorophyll *P. amabilis* plantlet by planting in Vacin & Went (VW) medium added with FA with various concentrations are presented in Table 1. The results of total dissolved carbohydrate content of P. amabilis plantlets by planting on Vacin and Went (VW) medium added with AF with various concentrations are presented in Table 2.

Table 1. The Chlorophyll Content of *P.amabilis* Plantlet Results of Induced With Fusaric Acid

Fusaric Acid Concentration (ppm)	Chlorophyll a Content (mg/g tissue)	Chlorophyll b Content (mg/g tissue)	Total Chlorophyll Content (mg/g tissue)
0 (control)	$0,05 \pm 0,003^{c}$	$0,07 \pm 0,003^{c}$	$0,12 \pm 0,006^{c}$
10	$0,26 \pm 0,019^{b}$	$0,12 \pm 0,018^{b}$	$0,38 \pm 0,025^{b}$
20	$0,24 \pm 0,027^{b}$	$0,13 \pm 0,005^{b}$	$0,37 \pm 0,022^{b}$
30	$0,29 \pm 0,017^{ab}$	$0,14 \pm 0,004^{ab}$	$0,43 \pm 0,016^{ab}$
40	$0,32 \pm 0,016^{a}$	$0,16 \pm 0,006^{a}$	$0,49 \pm 0,022^{a}$

Note: Numbers followed by the same letter, not significantly different at 95% confidence level

Tabel 2. Total Dissolved Carbohydrate Content of *P.amabilis* Plantlet Results of Induced with Fusaric Acid

Fusaric Acid Concentration (ppm)	Total Dissolved Carbohydrate Content (mg/g tissue)
0 (control)	$1,21 \pm 0,016^{c}$
10	$1,33 \pm 0,035^{b}$
20	$1,51 \pm 0,014^{a}$
30	$1,53 \pm 0,024^{a}$
40	$1,55 \pm 0,040^{a}$

Note: Numbers followed by the same letter, not significantly different at 95% confidence level

Chlorophyll is a very important part in a plant. Chlorophyll plays a role in the process of photosynthesis, with the main function of utilizing solar energy, and processing it into carbohydrates. In theory, healthy plants will continue to produce chlorophyll as plants age, but due to several factors the presence of chlorophyll will decrease. When all environmental factors are in the right conditions, the presence of chlorophyll will be very high in a plant. When the presence of chlorophyll in a plant is low, it can be explained that the presence of pathogens or plant-disturbing organisms that interfere with plant physiology. Increase or decrease in the value of chlorophyll content can indicate the level of resistance of a variety of downy mildew in maize [19]. The results of chlorophyll content analysis in this study are in line with research conducted by Andari and Nurcahyani [20] and Isharnani, *et al.* [21] showed an increase in the content of chlorophyll A, chlorophyll B, and total chlorophyll *Spathoglottis plicata* plantlet with increasing concentration of FA.

Cellulose is an important part in forming plant natural resistance, namely mechanical, structural and anatomical resistance. Cellulose is the main constituent of plant cell walls and the degree of rigidity is determined by lignin deposits. The high cellulose content has an effect on the increased cohesiveness of the leaf cell wall structure. This condition is very beneficial for plants, especially in preventing the penetration of fungal hyphae from penetrating the leaf cell walls until they reach the xylem [22].

4.Conclusions

Based on the results of the study, scaling resistance by using fusaric acid of 10, 20, 30 and 40 ppm can increase the content of chlorophyll a, chlorophyll b, total chlorophyll and total dissolved carbohydrate content *P. amabilis* plantlets, with the highest yield at 40 ppm fusaric acid concentration.

References

[1]. Rodica A, Bavaru A and Livia B 2011 Anatomical Aspects Of *Phalaenopsis Amabilis* (L.) Blume *Annals of RSCB* **16**(2)

[2]. Puspitaningtyas D M dan Mursida W 2010 Koleksi Anggrek Kebun Raya Bogor *UPT Balai Pengembangan Kebun Raya-LIPI Bogor* **1**(2)

[3]. Stephan W G, Gunter A F, Phillip J C And Michael F F 2018 Orchid Conservation: Bridging the Gap Between Scienceand Practice *Botanical Journal of the Linnean Society* **186** pp 425-434

[4]. Chung W C, Chen L W, Huang J H, Huang H C and Chung W H 2011 A New Forma Specialis of *Fusarium solani* Causing Leaf Yellowing of *Phalaenopsis Plant Pathology* **60** pp 244 - 252

[5]. Djatnika I 2012 Seleksi Bakteri Antagonis untuk Mengendalikan Layu Fusarium pada Tanaman *Phalaenopsis J.Hort* **22** (3) pp 276-284

[6]. Wedge D E and Elmer WH 2008 Fusarium Wilt of Orchids *ICOGO Bull* **2** (3) pp 161-168.

[7]. Nurcahyani E, Agustrina R and Handayani T T 2016 The Protein Profile of the Plantlets of *Spathoglottis plicata* Bl. Induced Resistance to *Fusarium oxysporum Journal of Plant Science* **4**(5) pp 102-105

[8]. Nurcahyani E, Agustrina R, Suroso E and Andari G 2016 Analysis of Peroxidase Enzyme and Total Phenol from Ground Orchid (*Spathoglottis plicata* Bl.) as Result of the In Vitro Fusaric Acid Selection Toward to *Fusarium oxysporum International Journal of Apllied Agricultural Science* **2**(6) pp 79-82

[9]. Nurcahyani E, Sumardi, Hadisutrisno B and Suharyanto E 2017 DNA Pattern Analysis of *Vanilla planifolia* Andrews Plantlet which Resistant to *Fussarium oxysporum* f. sp.*vanillae WJPLS* **3**(4) pp 27-34

[10]. Azhari A, Nurcahyani E, Qudus H I and Zulkifli 2018 Analisis Kandungan Prolin Planlet Jeruk Keprok Batu 55 (*Citrus reticulata* Blanco var. crenatifolia) setelah diinduksi Larutan Atonik dalam Kondisi Cekaman Kekeringan secara *In Vitro Analit: Analytical and Environmental Chemistry* **3**(01) pp 69-78

[11]. Rosyalina N, Nurcahyani E, Qudus H I and Zulkifli 2018 Pengaruh Larutan Atonik Terhadap Kandungan Karbohidrat Terlarut Total Planlet Jeruk Siam Pontianak (*Citrus Nobilis* Lour. var. Microcarpa Hassk.) secara In Vitro *Analit: Analytical and Environmental Chemistry* **3**(01) pp 61-68

[12]. Nurcahyani E, Sumardi, Irawan B, Sari E Y and Sari T L 2019 In Vitro Study: Induced Resistance Of Cassava (*Manihot esculenta* Crantz) Plantlet Against *Fusarium oxysporum* Based on Analysis of Phenol Content *WJPLS* **5**(2) pp 195-198

[13]. Bouizgarne B, Bouteau H E M, Frankart C, Reboutier D, Madiona K, Pennarun A M, Monestiez M, Trouverie J, Amiar Z, Briand J, Brault M, Rona J P, Ouhdouch Y and Hadrami E I 2006 Early Physiological Responses of *Arabidopsis thaliana* Cells to Fusaric Acid : Toxic and Signallling Effects *New Phytologist* 169 pp 209-218
[14]. Nurcahyani E, Sumardi I, Hadisutrisno B dan Suharyanto E 2012 Penekanan Penyakit Busuk Batang Vanili (*Fusarium oxysporum* f.sp. vanillae) melalui Seleksi Asam Fusarat secara In Vitro *Jurnal Hama dan Penyakit Tumbuhan Tropika JHPTT* **12**(1) pp 12-22
[15]. Dehgahi R, Latiffah Z, Azhar M, Alireza J and Sreeramanan S 2015 Effects of Fusaric Acid Treatment on The Protocorm-like Bodies of *Dendrobium sonia*-28 *Springer* **253**(5) pp 1373-1383
[16]. Gross J 1991 *Pigmentin Vegetable, Chlorophyl and Caretinoids* (New York: Van Nonstrand Reinhold)
[17]. Miazek Mgr Inz 2002 Kristian Chlorophyl Extraction from Harvested Plant Material *Supervisor Prof Dr Ha Inz Stainslaw Ledakowicz*
[18]. Witham D and Robert M 1993.*Exercise in Plant Physhiologi Second Edition* (Boston: Prindle Weber and Scimdt)
[19]. Agustamia C, Ani W dan Christanti S 2016 Pengaruh Stomata dan Klorofil pada Ketahanan Beberapa Varietas Jagung terhadap Penyakit Bulai *Jurnal Perlindungan Tanaman Indonesia* **20**(2) pp 89-94
[20]. Andari G dan Nurcahyani E 2018 Analisis Kandungan Klorofil Hasil Ketahanan Terimbas *Fusarium Oxysporum* terhadap *Spathoglottis plicata* secara In Vitro *Musamus Journal Of Animal Livestock Science* **1**(1)
[21]. Isharnani E C, Nurcahyani E and Lande M L 2015 Chlorophyll Content of Leaves of Planlet Ground Orchid (*Spathoglottis plicata* Blume) Result of Induced Resistance of the In Vitro Fusaric Acid *Prosiding Seminar Nasional Swasembada Pangan Politeknik Negeri Lampung* ISBN 978-602-70530-21 pp 86-92
[22]. Kesten C, Menna A and Rodrı´guez C S 2017 Regulation of Cellulose Synthesis in Response to Stress *Current Opinion in Plant Biology* **40** pp 106–113

Acknowledgement
Thanks the authors to the Institute for Research and Community Service through the BLU fund of University of Lampung, based on the Letter of Assignment of "**PENELITIAN PASCASARJANA**" 2019 Number of Contract: 1921/UN26.21/PN/ 2019/26 Juni 2019.