Analysis Resistance of Planlet *Phalaenopsis amabilis* (L.) Bl. Results of Induced Resistance to Fusarium Withered Disease In Vitro

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ABSTRACT

Phalaeonopsis amabilis is one type of orchid that is famous for its beauty. In its growth *P. amabilis* experienced problems such as the emergence of fungal pathogens. *Fusarium oxysporum (Fo)* is a fungus that can cause fusarium wilt in *P. amabilis*. Control to prevent fungal infections one of them with the mechanism of impact or induced resistance.Fusarium wilt selection can be done using Fusaric Acid (FA). This study aims to determine the effective concentration of AF to induce fusarium wilt in *P. amabilis* planlets and analyze the resistance of *P. amabilis* planlets to *Fo* in vitro. This study uses a Completely Randomized Design (CRD) with one factor, namely FA concentration consisting of 4 levels, namely 0 ppm (control), 20 ppm, 40 ppm and 60 ppm, each concentration is done 5 times. The results showed that the effective FA concentration to induce fusarium wilt in *P. amabilis* plantlet was 60 ppm concentration. The resistance of *P. amabilis* plantlets to *Fo* in vitro using FA is 60 ppm concentration because it is able to fusarium wilt disease well with resistant criteria.

Keywords: *Fusaric Acid*, *Fusarium oxysporum*, *Induced Resistance*, *In Vitro*, *Phalaenopsis amabilis* (L.) Bl.

I. Introduction

Orchid is an ornamental plant that is very popular and much preferred to be cultivated, especially the people of Indonesia (Indayanti, 2014). *Phalaenopsisamabilis* is one of the most popular orchids with diversity and beauty of the flowers (*Fauziah et al.*, 2014). *P. amabilis* has been established as a charm of Indonesian charm (Widiarsih and Dwimahyani, 2013). 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 (*Rafflesiaarnoldii* R. Br.) as a rare puspa (Puspitaningtyas and Mursidawati, 2010).

In its growth, *P. amabilis* has encountered problems such as the emergence of diseases from fungal pathogens, bacteria, or viruses that attack parts of the body of orchid plants (Djatnika, 2012). The pathogenic fungus that most attacks orchids is *Fusarium*

oxysporum (*Fo*) (Chung *et al.*, 2011). In orchids, *Fo* will cause fusarium wilt. Fusarium wilt in orchids will show symptoms in the form of leaves and stems yellowing, wrinkled, thin and curved, decomposed leaf neck reaches the base of the stem (Soelistijono, 2015). Control of airborne infectious pathogens by inoculation in plants with various biological agents causes increased resistance to subsequent inoculation by the main pathogen. One type of biological control is the mechanism of induced resistance or induced resistance (Agrios, 2005).

The use of fusaric acid as a screening agent in in vitro selection can produce mutant cells or tissue that are insensitive to fusaric acid, so that after regeneration into plants can produce strains that are resistant or tolerant of pathogenic infections. Disease control by this alternative method does not cause negative impacts, as it does in the use of fungicides by using superior varieties that are resistant to *Fusarium oxysporum* (Nurcahyani*et al.*, 2012).

In vitro selection of resistance to *Fusarium* sp. in several plant species was carried out using pure toxin, fusaric acid. This method has been used in breeding Kepok bananas (Damayanti, 2010), Vanilla (Nurcahyani*et al.*, 2012), *Spathoglottisplicata* (Nurchayani*et al.*, 2016), Cassava (Nurcahyani*et al.*, 2019) Phalaenopsis (Nurcahyani*et al.*, 2020) and Markisa (Flores *et al.*, 2012). The study showed a positive correlation between symptoms and disease events due to fusaric acid treatment in vitro.

II. Material and Methods

The tools used in this study areautoclave, *Laminar Air Flow* Cabinet (LAF), spectrophotometer, a 250 ml culture bottle, 100 ml and 500 ml volume measuring cups, 10 cm diameter petri dishes, aluminum foil, tweezers, scalpels, scalpel blades, micropipets, test tubes, test tube racks, hot plates, Ohaus analytical scales, petridish, tissue, paper label and camera.

The materials used in this study were the *Phalaenopsisamabilis* (L.) Bl. , pure fusaric acid produced by Sigma Chemical Co. {Fusaric acid (5-butylpicolinic acid) from Giberellafujikuroi}, 96% alcohol, sucrose, Hydrochloric Acid (HCl), Potassium Hydroxide (KOH), and the Vacin and Went medium.

Procedure

The medium used is Vacin and Went (VW), the medium is sterilized for 15 minutes. The sterilized VW medium is then added FA with a concentration of 0 ppm (control), 20 ppm, 20 ppm, and 60 ppm for disease resistance selection.The explants used were sterile plantlets.Planlets from culture bottles were removed with sterile scalpels and one by one

placed on a 10 cm diameter petri dish, furthermore plantlets were planted in each culture bottle containing the specified treatment medium. Each concentration was done 3 times and each repetition consisted of 2 *P. amabilis*explants in each culture bottle.

Monospora inoculation was carried out according to the research technique (Nurcahyani*et al.*, 2012) as follows. *Fo* inoculation was carried out directly on *P. amabilis*plantlets in culture bottles. The fungal microconidium with 1.7 x 10^4 spore density per mL was dripped on 1-2 drops plantlets. Furthermoreincubated at room temperature (25 ˚C) for 24 hours. Observation was carried out for 3 weeks by observing and counting the number of leaves that showed symptoms of wilting with the withered index according to He*et al.*, (2002) as presented in Table 1.

Disease intensity (DI) is calculated by the formula:

$$IP = \frac{\sum(n \times v)}{N \times Z} x\ 100\ \%$$

Information :
IP = Pathogenic Intensity
n: Number of plants on score v
N: Number of plants tested
Z: Highest score

Table 1
Withered index according to He *et al.*, (2002)

Score	Information
0	No yellow symptoms (wilting or healthy plants)
1	1-2 yellow leaves (withered)
2	3 yellow leaves (withered)
3	4 yellow leaves (withered)
4	4 More than 4 yellow leaves (withered) or dead plants

The level of plant resistance is determined based on scoring with
referring to the provisions of Wibowo (2002) as shown in Table 2 as follows.

Table 2 Crop Resistance Levels according to Wibowo (2002)

IP	Endurance Criteria
≤ 25	Sensitive
25 <IP ≤ 50	Moderate
> 50or die	Resistant

Note: IP = Pathogenic Intensity

III. Results and Discussion

The results of *P. amabilis* plantlet selection based on the percentage of the number of live plantlets with various concentrations of Fusaric Acid (FA) are presented in Table 3.

Table 3.Percentage of the number of live plantlets selected by fusaric acid.

Fusaric Acid Concentration (ppm)	**Percentage of number of life planlets per week** (%)			
	I	II	III	IV
0	100	100	100	100
20	100	100	100	100
40	100	100	100	90
60	100	100	100	80

Based on Table 3, it can be seen that the addition of FA at concentrations of 0 ppm (control), 20 ppm, 40 ppm and 60 ppm in observations of the first week to the third week showed the number of plantlets that live reached 100%. Plantlets with concentrations of 40 ppm and 60 ppm in the fourth week experienced death with a percentage of 10% and 20% marked on the yellow stems and leaves.

Based on the results of the study showed the influence of FA on *P. amabilis* plantlets planted by selection on medium in vitro. The effect of FA is seen at the fifth week at concentrations of 40 ppm and 60 ppm. The results of research supported by Damayanti (2010) announced that the higher concentration of fusaric acid can reduce the percentage of shoot life

Testing of *P. amabilis* plantlets against *Fo* was carried out after the plantlets were selected with FA for 4 weeks, furthermore the plantlets were subcultured into VW media, after obtaining plantlets that were resistant to FA then tested their resistance to fungi to determine the resistance of *P. amabilis* plantlets by calculating the percentage of leaves wilted or yellow are presented in table Table 4

Table 4. Percentage of yellow or yellow withered leaves in each fusaric acid treatment.

Treatmen	**Percentage of wilted or yellow leaves on the day of observation to:**					
	0	**4**	**8**	**12**	**16**	**20**
0	0	33.33	33.33	33.33	66.67	66.67
20 ppm	0	0	33.33	33.33	66.67	66.67
40 ppm	0	0	0	33.33	33.33	33.33
60 ppm	0	0	0	0	0	0

Based on table 4, it is known that wilting symptoms that appear on day 4 after inoculation, can be seen at a concentration of 0 ppm (control) with an average percentage of 33.33%, while at a concentration of 20 ppm symptoms of wilting are seen on the 8th day with average - 33.33%. The percentage of wilted leaves increased at concentrations of 0 ppm and 20 ppm on the 20th day with an average of 66.67%. At a concentration of 40 ppm withering symptoms appeared on the 12th day with an average of 33.33%, whereas at a concentration of 60 ppm it did not show any wilted or yellow leaves until the last observation. According to Nurcahyani*et al.* (2012) stated that the effect of fusaric acid concentration as a scavenger material was able to reduce the percentage of wilting or yellow leaves.

Based on the scoring of the symptoms of wilting or yellow leaves that emerge, it can be seen the percentage of disease intensity and endurance criteria of each AF treatment are presented in Table 5.

Table 5.Disease intensity of endurance test results and level of resistance of *P. amabilis*planlets in the FA treatment.

Treatmen	**Day of Observation**									
	4		8		12		16		20	
	IP (%)	Criteria Resistance	IP (%)	Criteria Resistance	IP (%)	Criteria Resistance	IP (%)	Criteria Resistance	IP (%)	Criteria Resistance
0 ppm	33.33	Moderate	33.33	Moderate	33.33	Moderate	66.67	Susceptible	66.67	Susceptible
20 ppm	33.33	Moderate	33.33	Moderate	33.33	Moderate	33.33	Moderate	66.67	Susceptible
40 ppm	0	Resistant	0	Resistant	33.33	Moderate	33.33	Moderate	33.33	Moderate
60 ppm	0	Resistant	0	Resistant	0	Resistant	0	Resistant	0	Resistant

Information.IP = Pathogenic Intensity

Based on Table 5, it can be seen that the highest disease intensity is shown at a concentration of 0 ppm (control) and a concentration of 20 ppm on the 20th day with a disease intensity reaching an average of 66.67% so that it can be declared susceptible to fusarium wilt disease, while at a concentration of 40 ppm shows the intensity of the disease reaches an average of 33.33% on the 20th day so that the criteria for resistance are moderate in fusarium wilt disease. At a concentration of 60 ppm the 20th observation showed criteria of endurance with 0% disease intensity so as to be able to fuse Fusarium wilt in *P. amabilis* well. *P. amabilis* plantlets aged 20 days after inoculation with *Fo* presented in Figure

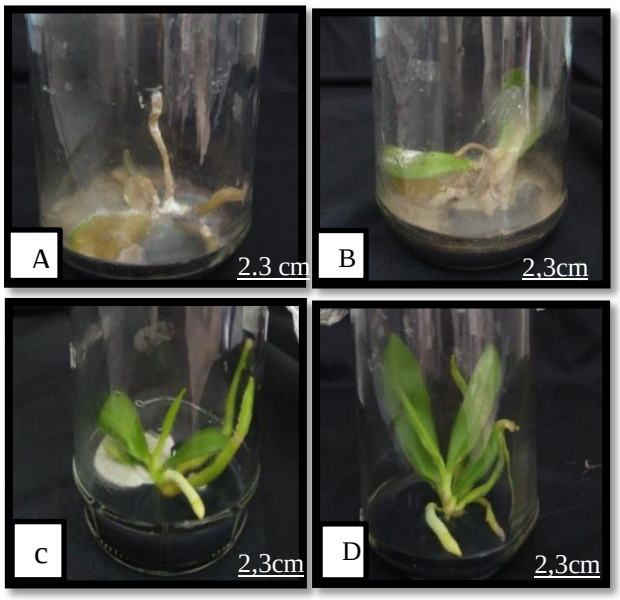

Figure 1. Results of *Fo* inoculation on *P. amabilis* plantlets aged 20 days after FA treatment
A = 0 ppm (control)
B = Planlet*P. amabilis* resulting from FA scaling of 20 ppm
C = Planet *P. amabilis* results from the FA scaling result of 40 ppm
D = Planlet*P. amabilis* resulting from FA scaling of 60 ppm

Typical symptoms of fusary rot disease generally begin to appear in the 1st to 3rd week after inoculation, starting with the rot of the base of the stem and leaves become yellowish (Sukmadjaja *et al.,* 2003). Young plants are more susceptible to pathogens and virulent pathogens with environmental conditions that are suitable for the development of pathogens causing greater disease like lihood (Agrios, 2005).

Conclusion

The concentration of fusaric acid that is effective for fusarium wilt disease in *P. amabilis*plantlet is a concentration of 60 ppm. The resistance of *P. amabilis* plantlet to *Fusariumoxysporum* in vitro uses fusaric acid which is a concentration of 60 ppm that is resistant to fusarium wilt. Concentration of 60 ppm can affect fusarium wilt disease with a disease intensity of up to 0%, which is the resistant criteria.

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