In Vitro Selection *Phalaenopsis amabilis* (L.) Bl. Plantlets Result of Induced Resistance with Fusaric Acid

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Abstract: *Phalaenopsis amabilis* (L.) Bl. is an original orchid from Indonesia and one of Indonesia's national flowers, known as "Puspa Pesona", is included in the list of endangered species. *P. amabilis* is also one of the most popular orchid plants for various groups of people because of the beauty of the shape and color of flowers, but the production of *P. amabilis* in Indonesia is still far behind compared to other countries such as Thailand, Taiwan, Singapore and Australia. Fusarium wilt is caused by *Fusarium oxysporum* (*Fo*) which until now has not been able to be overcome effectively. The use of *P. amabilis* plantlets that are resistant to Fusarium wilt is expected to be an important alternative for disease control. The purpose of this reserach was to study and determine the concentration of Fusaric Acid (FA) in the selection of *P. amabilis* plantlets that were tolerant of Fusarium wilt. This study used *P. amabilis* plantlets with 5 levels of FA concentration, namely 0 ppm, 10 ppm, 20 ppm, 30 ppm, and 40 ppm. The results showed that the concentration of FA tolerant for optimum growth was 40 ppm. The results of in vitro selection with subcultured FA on multiplication medium resulted in a number of live *P. amabilis* plantlets is 100% (10 -30 ppm), which were insensitive to FA, whereas at 40 ppm a number of live *P. amabilis* plantlets is 60%.

Key words: *Fusarium oxysporum*, *Induced Resistance*, *Phalaenopsis amabilis*, *In vitro*

INTRODUCTION

Orchid is an ornamental plant that is very popular in the community, not less than 5000 species live in the wilds of Indonesia [1]. orchid have a variety of shapes, colors and sizes of flowers, thus creating a special attraction for orchid lovers, as well as being a plant that has quite high economic value and relatively stable prices [2,3]. The most popular type of orchid on the market is *Phalaenopsis amabilis* or known as the moon orchid [4]. The moon orchid is one of Indonesia's national flowers established by Presidential Decree No. 4/1993, as Puspa Pesona, besides jasmine (*Jasminum sambac* L.) as the nation's puspa, and giant padma flowers (*Rafflesia arnoldii* R. Br.) as a rare puspa [5]. Promising economic value makes the moon orchid much hunted in nature that threatens its sustainability, so it is included in the CITES Appendix II list [6], so it needs to be supported by the production of quality orchid seeds.

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* [7]. *Fo* causes fusarium wilt which interferes with the growth of orchids [3]. 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 [8].

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 [9,10,11,12,13,14]. 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 [9,10,15,11,14].

Fusaric acid (FA) is a metabolite produced by several species of fungi from the genus Fusarium. FA chemically called 5-n-butylpicolinic acid. This acid can be toxic (concentrations of more than 10^{-5} M) so as to inhibit the growth and regeneration of cultures [16,17], but at non-toxic concentrations (below 10^{-6} M) it

actually helps to induce phytoalexin synthesis, a form of plant response to inhibit pathogenic activity [17]. Several parameters can illustrate the mechanism of plant resistance to pathogenic infections including an increase in phenol compounds, an increase in peroxidase enzymes (including the PR-protein group), and the presence of lignification [18,19,20].

The use of FA in tolerant concentrations so far has never been reported with certainty and accuracy in the induction of the resistance (Induced Resistance) of *P. amabilis* plantlet against *Fo*. Therefore, research on the role of FA as an endurance inducer in vitro needs to be done. Control of Fusarium wilt in *P. amabilis* with FA to the best of the author's knowledge has never been done and unknown tolerant FA concentration for selection of *P. amabilis* plantlet with optimum growth.

METHODS

This research was held in March 2019 until July 2019 in the In Vitro Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung. The research material was a moon orchid plantlets [*Phalaenopsis amabilis* (L.) Bl.] which was affected by FA.

Preparation for Planting Medium and Selection

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), 10 ppm, 20 ppm, 30 ppm, and 40 ppm for disease resistance selection.

Planting of Plantlets in FA Selection Medium

The explants used were sterile plantlets. Plantlets from culture bottles were removed with sterile scalpels and one by one placed on a 10 cm diameter petri dish, then plantlets were planted in each culture bottle containing the specified treatment medium. Each concentration was carried out 5 replications and each replication consisted of 2 *P. amabilis* planlets in each culture bottle.

Percentage of Number of Living Plantlets and Plantlets Visualization

Includes the color of plantlet after being given FA treatment with the following classification: green, green with certain parts brown and brown.

Data Analysis

Data obtained from the growth of *P. amabilis* plantlets during selection with FA in the form of qualitative data and quantitative data. Qualitative data is presented in the form of comparative descriptive and supported by photographs. Quantitative data were tabulated with different concentration factors with 5 replications each treatment.

RESULTS AND DISCUSSION

P. amabilis is a plant with a high level of disease intensity, one of which is Fusarium wilt disease. This disease is caused by the fungus *Fusarium oxysporum* f.sp. lycopersici (Sacc.). This fungus is a soil-borne pathogen that can survive for a long time in the form of chlamidiospores even though there are no host plant [21]. Fusarium wilt is a very important and economically harmful disease because until now there has been no effective control [22]. One way to get a *P. amabilis* plantlet which resistant Fusarium wilt is to use FA. Induced resistance using FA is one of the biological control methods used to control Fusarium wilt.

Percentage of Live Plantlets and Plantlets Visualization

Observation of *P. amabilis* plantlets which planted on Vacin and Went (VW) medium with FA treatment at five concentration levels, namely 0 ppm (control), 10 ppm, 20 ppm, 30 ppm, and 40 ppm are presented in Table 1. The selection results show that the plantlets were still able to survive up to a concentration of 30 ppm, but at the 4th week with FA treatment of 40 ppm there were 4 plantlets died.

TABLE 1. Percentage of Live Plantlets Result of Selection with Fusaric Acid

Fusaric Acid Concentration (ppm)	Percentage of Live Plantlets on the Weeks- (%)			
	I	II	III	IV
0 (control)	100	100	100	100
10	100	100	100	100
20	100	100	100	100
30	100	100	100	100
40	100	100	100	60

Table 1 shows that for observation of weeks 1 to 3 in all FA treatments, the percentage of the number of *P. amabilis* living plantlets reached 100%. *P. amabilis* plantlets at the 4th week treated with FA 10 ppm, 20 ppm, 30 ppm, and the control did not experience death, but at a concentration of 40 ppm, 40% mortality occurred marked by the roots and leaves are brown.

The result of observations on *P. amabilis* plantlets showed the effect of giving FA which planted on in vitro selection medium. The result of this study are supported by [23] which states that there is a change in color to brown on plantlets given a high concentration of FA, while the highest percentage of live plantlets is shown on plantlets with lower concentration of FA.

The observation of *P. amabilis* plantlets at week 1 there has not been any decrease in the visualization percentage of *P.amabilis* plantlets, shown from 100% live plantlets at FA concentrations of 0 ppm, 10 ppm, 20 ppm, 30 ppm, and 40 ppm and are visually colored green. The 2nd week of the plantlet with a concentration of 40 ppm FA showed a decrease in the percentage of visualization that seen on the base of leaf on the *P. amabilis* plantlet to turn green brown. The decrease in visualization percentage at weeks 3 and 4 is presented in Figure 1, seen in FA concentrations of 30 ppm in the leaves and roots of plantlets turning green brown and at FA concentrations of 40 ppm there is 40% of the plantlets change to brown and dead at weeks 4. Visualization of *P. amabilis* plantlets was observed from week 1 to week 4. Results of *P. amabilis* plantlets selection with various FA concentrations based on the percentage of plantlets visualization are presented in Table 2.

TABLE 2. Percentage of Visualization Plantlets Result of Selection with Fusaric Acid

Fusaric Acid Concentration (ppm)	Percentage of Visualization Plantlets on the Weeks- (%)			
	I	II	III	IV
0 (control)	G : 100	G : 100	G : 100	G : 100
10	G : 100	G : 100	G : 100	G : 100
20	G : 100	G : 100	G : 100	G : 100
30	G : 100	G : 100	G : 80 GB : 20	G : 60 GB : 40
40	G : 100	G : 60 GB : 40	G : 50 GB : 50	G : 20 GB : 40 B : 40

Note: G= Green; GB= Green Brown; B= Brown

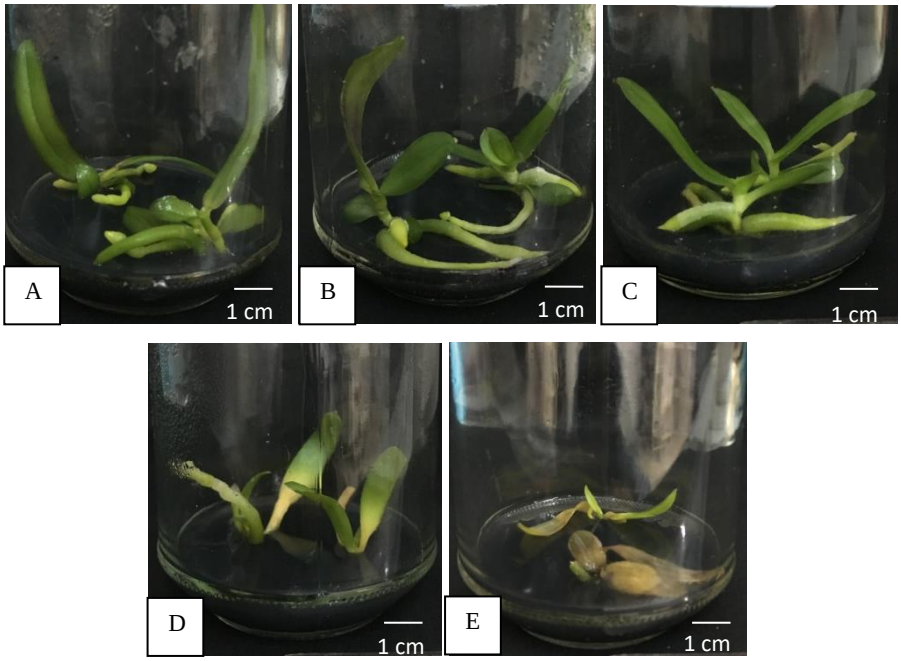

FIGURE 1. Development of *P.amabilis* plantlets after 4 weeks at various concentrations of FA. A = 0 ppm (control), B = 10 ppm, C = 20 ppm, D = 30 ppm, and E = 40 ppm.

Morphological characters of plantlets were seen to change in each treatment after 4 weeks giving of FA. Changes in plantlets occur from green to brown green and brown. According to [24] states that the change in color to brown on the *P. amabilis* plantlet is caused by an increase in phenolic compounds which is followed by oxidation from the activity of the enzyme oxidase (PPO). The selection results showed that the highest FA concentration of 40 ppm has occurred the selection process with 40% dead plantlets, therefore 60% of plantlets that survive and live was a selected plantlet with FA.

CONCLUSION

The optimum of FA concentration for in vitro selection of *P. amabilis* plantlets is 40 ppm. Selection results live plantlets in 100% (10-30 ppm) and 60% (40 ppm).

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