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INDUCE RESISTANCE OF *SPATHOGLOTTIS PLICATA* BL. TOWARD TO *FUSARIUM OXYSPORUM*

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ABSTRACT

Spathoglottis plicata attracts many people as an ornamental plant for parks, offices and housing complexes in urban areas. The most production constrain on Ground Orchid (*Spathoglottis plicata* Bl.) plantation recently has been caused by fusarium wilt caused by *Fusarium oxysporum* and until now still can not be solved effectively. In general, Indonesian farmers cope with fusarium wilt disease by using pesticides that often cause environmental pollution, while orchids are always physically close to the fans. Therefore, it should be an effective and environmentally friendly alternative. One of the most secure and efficient alternative diseases control for environment is by using resistant varieties. Ground orchid plantlets that are resistant to *Fo* have been selected by in vitro selection on Vacin & Went (VW) medium containing fusaric acid (FA) at different concentrations. The objectives of this research was to determine: Resistance criteria of *S. plicata* plantlet that was induced by fusaric acid on *F. oxysporum* infection through in vitro selection. This research was conducted at In Vitro Laboratory of Dept. of Biology, Faculty of Mathematics and Natural of Science, University of Lampung, Bandar Lampung, Indonesia. The research was compiled by using Completely Randomized Design (CRD) with one factor that is FA consisting of 5 levels: 0 ppm, 10 ppm, 20 ppm, 30 ppm, and 40 ppm on WV medium. Data analysis used ANOVA (analysis of varian) at significance level 5% and a further test with LSD (Least Significant Difference) at the significance level 5%. The result showed that: The resistance criteria of *S. plicata* plantlet on day 28 (0 ppm) was control and 10 ppm was susceptible. At 20 ppm and 30 ppm, its resistance criteria were moderate. At concentration of 40 ppm, its resistance criteria was resistant.

Keywords: *Spathoglottis plicata*, *Fusarium oxysporum*, Induced Resistance, Fusarium wilt, Fusaric Acid

I. INTRODUCTION

Orchid is a flowering plant that has economic value is high enough and the price is relatively stable. Ground orchid (*Spathoglottis plicata* Bl.) is one of orchids is much preferred. One of the obstacles encountered in the cultivation of *S. plicata* is the presence of pathogenic fungi which can attack several parts of plants such as stems, leaves or roots (Djatnika, 2012). Fusarium wilt caused by the fungus *Fusarium oxysporum* is an important disease and one of the obstacles in the quality and production of *S. plicata* (Palmer, 2011).

One alternative way to control the disease that is safe, efficient and effective, and safe for the environment, among others, using varieties that are resistant. The development of *S. plicata* resistant cultivars can be done by in vitro selection method that is explants form of tissue or organ in a medium containing fusaric acid (FA) that selective concentration (Bouizgarne et al., 2006). Use of FA as a selective agent in vitro selection may produce mutant cell or tissue which is insensitive to FA, so that after being regenerated into plants can produce resistant strains of pathogen infection (Arai and Takeuchi, 1993).

II. MATERIALS AND METHODS

Materials used are plantlets of *Spathoglottis plicata* Bl. aged 6 months were obtained from the personal collection of Dr. Endang Nurcahyani, pure Fusaric Acid manufactured by *Sigma chemical Co*. {Fusaric acid (5-butylpicolinic acid) from Giberella fujikuroi}, 70% alcohol, distilled water,

Benzine Amino Purine (BAP), indole-3-Acetic Acid (IAA), sucrose, Potassium Hydroxide (KOH), acid chloride (HCl) and chemicals medium Vacin & Went (VW) solid.

Research compiled by using a completely randomized design (CRD) with one factor, FA concentration which consists of 5 levels: 0 ppm, 10 ppm, 20 ppm, 30 ppm and 40 ppm. Each concentration was repeated 5 times and each replication consisted of three explants *S. plicata* in each culture bottle. Then testing the resilience of plantlets of *S. plicata* against *F. oxysporum*.

Medium VW on a sterilized bottle culture coupled with a concentration corresponding FA treatment. Fusaric acid before use, diluted with distilled water to obtain the concentrations specified, then filtered using a syringe filter having a diameter of 0.45 µm, was done 2 times and filter diameter of 0.22 µm was done one time. Filtering is done in a sterile room in the Laminar Air Flow (LAF) Cabinet. Furthermore, FA is added to VW medium. Before use, the medium was incubated for 7 days at room temperature (25 °C) to ensure that FA has been pre-screened. If within 7 days of no contamination on the medium, the medium can be used.

S. plicata plantlets are planted in the VW medium in the Laminar Air Flow (LAF) Cabinet. Plantlets from culture bottle issued with a sterile scalpel and one by one placed on a petridish, diameter of 10 cm, then plantlets sorted one by one, after it is planted on each bottle culture medium. Each concentration is done five replications and each replication consisted of three explants of *S. plicata* in each culture bottle. Selection plantlets carried out for 30 days_At the end of the 4th week, were evaluated to determine the concentration of FA are tolerant to the selection of *S. plicata* plantlets in vitro.

Fusarium oxysporum inoculation was performed directly on the plantlets in the culture bottles (Hadisutrisno, 1995). Mikrokonidium fungus *F. oxysporum* with a density of 1.7 x 104 spores per ml is dripped onto the plantlets 1-2 drops, then incubated at room temperature (25°C) for 24 hours. Observations were made starting on day 3rd after inoculation for four weeks by observing and counting the number of leaves that show symptoms of wilting index by He et al (2002). Disease Intensity (IP) is calculated according to the formula of Wibowo (2002).

$$DI = \frac{\Sigma(n \times v)}{N \times Z} \times 100\%$$

The level of plant resistance is determined by scoring with reference to the provisions of Wibowo (2002).

DI (%)	Criteria of Resistance
≤ 25	Resistant
25 < DI ≤ 50	Moderate
>50 or die	Susceptible

Information : DI = Disease Intensity

III. RESULT AND DISCUSSION

Fusarium wilt disease caused by *F. oxysporum* is an important disease that is one of the obstacles to the growth of orchids (Palmer, 2012). In orchids, fusarium wilt caused the death of more than 50% of the number of plants grown orchid (Wedge & Elmer, 2008).

Observation of wilting symptoms on the leaves of plantlets which is done every day for 4 weeks showed symptoms of wilting on *S. plicata* orchid plantlets in the culture bottle. Furthermore, based on the scoring of the symptoms that appear wilted or yellow can be determined intensity of the disease (DI) and the criteria for each treatment resistance.

Criteria of resistance on treatment outcome plantlets control and the provision of 10 ppm produced plantlets resistance that criteria are susceptible to the disease intensity at 91% and 83%, the highest disease intensity shown by the control that is 91%. Induced resistance to FA at concentrations of 20 and 30 ppm produces plantlets that have the disease intensity reaches 33%, so the durability criteria

was moderate. In the treatment of 40 ppm FA there are no symptoms of the disease that is resistant durability criteria.

Based on data from the disease intensity and endurance above it can be seen that FA treatment of 40 ppm able to induce the most good resistance so as to reduce the intensity of the disease up to 0% and raising the criteria become resistant. The above shows that FA is able to induce resistance orchid plantlets of *S. plicata* against fusarium wilt. The results of this study supported the opinion of Arai and Takeuchi (1993) which describes the correlation between plant resistance to the toxin with disease resistance, so that pure toxin FA can be used as a component selection.

This result is in line with research conducted by Nurcahyani *et al.* (2012) which states that Induced resistant of vanilla against fusarium wilt can reduce the intensity of the disease reached 25% at 110 ppm treatment. The results also support the statement Agrios (2005) which states that the expression of induced resistance is by decreasing the intensity of the disease.

IV. CONCLUSION

Based on the results of research and discussion above, it can be concluded that the criteria for resistance plantlets of *S. plicata* on day 28 (controls) and 10 ppm is vulnerable. At 20 ppm and 30 ppm which is a moderate resistance criteria. At a concentration of 40 ppm resistance criteria are resistant to disease intensity to 0%.

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