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Analysis of The Reducing Sugar of Cassava (*Manihot esculenta* Crantz.) Mutant Plantlets Resistant to Fusarium Wilt.

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Abstract. Indonesia is the second country producing Cassava (*Manihotesculenta* Crantz) after Nigeria. One of the Indonesian Province, Lampung, is a Cassava central land in Indonesia, with a total production of 7,39 million tons in 2015. However, there are still many Cassava cultivation production constraints, such as Fusarium wilt disease. The disease is caused by the fungus *Fusariumoxysporum*, which until now still cannot be effectively addressed. So far, the farmers have eradicated the fungus by using fungicides, which have adverse impacts on the environment. Therefore, it's necessary to control the disease biologically by using a superior variety resistant to *Fusariumoxysporum*. Fusaric acid is a toxin produced by *Fusariumoxysporum* fungus which is mostly found in vitro selection in plants. Disease resistance can be obtained by induced resistance. The purpose of this study is to analyze the content of the reducing sugar of the Cassava plantlet after induced fusaric acid in vitro. The research using Completely Randomized Design, of one factor, with fusaric acid concentrations, consists of 5 levels 0 ppm, 60 ppm, 80 ppm, 100 ppm, dan 120 ppm in Murashige and Skoog medium. The data analysis used Analysis of Variance with a 5 percent significance level. The results of this study indicate that the increased concentration of fusaric acid, then also increases the reducing sugar content in the *Fusariumoxysporum* resistant Cassava plantlet.

INTRODUCTION

Cassava (*Manihotesculenta* Crantz.), is the third most important crop in the world and a staple food source and also become an income throughout the tropics. Cassava cultivation can be a livelihood for more than 500 million farmers [1]; [2]. Cassava is an important food commodity in Indonesia. In the future, this commodity will have a more strategic role in the lives of the people and the country's economy. Based on the area of the harvest of food commodities, cassava is on the third rank after rice and corn, which are the three main sources of carbohydrates in the community [3].

One of the problems encountered in cultivating Cassava is Fusarium wilt caused by the fungus *F.oxysporum* (*Fo*) and is medium-transmitted.[11,12] It has been reported that 50% of Cassava tubers produced and harvested in Nigeria were lost due to disease. The main causes of the decay of Cassava include *Aspergillusflavus*, *Aspergillusniger*, *Botryodiplodiatheobromae*, *Collectotrichumspp*, *Geotrichumcandidum*, *Penicilliumchrysogenum*, *Pennicilliumdigatum*, *Fusariumoxysporum.*[13,14,15] This organism reduces the quantity and quality of the plant tubers [16]

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Indonesia is the second-largest producer of Cassava after Nigeria, with an average total supply of five years of 9.67 million tons or 10.61% of the total world cassava supply, followed by Brazil, India, and the United Republic of Tanzania each ranging from 8.67 - 4.96 million tons or 9.52% - 5.44%, with the rest contributing below 5.30% [4].

The Central Statistics Agency noted that the Cassava land center in Indonesia was controlled by Lampung province with a harvest area of 324,100 ha in 2012. In 2013, cassava production in Lampung reached 8.33 million tons and in 2015 it was 7.39 million tons. This situation makes Lampung as a supplier of one-third national Cassava production from national production of 23.92 million tons [5]. However, there are still many production constraints in Cassava cultivation, including Fusarium wilt disease. This disease is caused by the fungus *Fusariumoxysporum* (*Fo*) which until now still can't effectively be treated.

One of the alternatives methods to control the disease that is efficient, effective, and safe to the environment is the resistant varieties. The use of high yielding varieties that are resistant to high yielding fo is one of the important alternative disease control that doesn't cause negative impacts. Development of Fo resistant cassava varieties can be carried out by in vitro selection methods, namely culturing explants in the form of tissue or organs in a medium containing selective fusaric acid concentration [6];[7]; [8].

Research on Cassava Induced Resistance with fusaric acid (FA) has been carried out previously. They found out indications of tolerant FA concentration for the selection of in vitro resistant plantlets. Inoculation of *Fusariumoxysporum* (*Fo*) fungi isolates in resistant Cassava was carried out in vitro, followed by the analysis of DNA patterns compared with controls. The outcome of the study, in the form of a Cassava mutant with a new (specific) DNA band measuring 550 bp (OPA_1) and 300 bp (OPA_10), was predicted as an RAPD marker candidate for Cassava's resistance to *Fo* [17].

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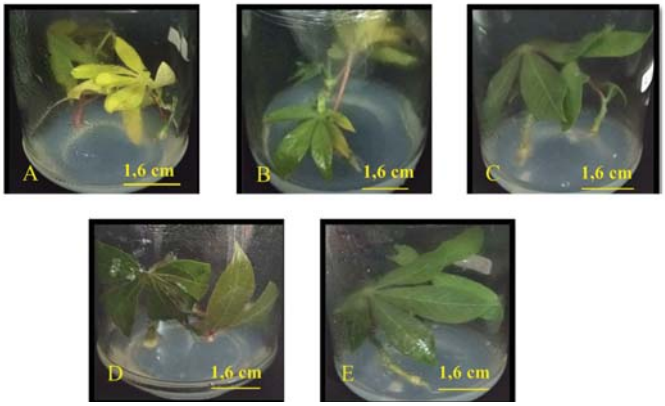

FIGURE 1. Control Cassava Planlets (A) and Cassava mutant plantlets treated with various concentrations of fusaric acid: B.60 ppm;C.80 ppm; D. ppm; and E.120 ppm.

METHOD

This research was conducted from April 2020 to July 2020 at the In Vitro Culture Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, University of Lampung.

Analysis of changes in sugar reduction level on the substrate is using the Luff Schoorl method. Five grams of the fermented substrate are taken from the bottle every 24 hours, then added with 50 mL of distilled water and stirred evenly. The suspension was centrifuged at 4000 rpm for 20 minutes, and the supernatant is used to test the reducing sugar levels. Put pipette 10 mL supernatant into a boiling flask, then pour 10 mL of Luff Schoorl reagent. The sample was boiled on reflux for 10 minutes, then carefully added 6 mL KI 20% and 10 mL H2SO4 through the pumpkin wall. Titrate the sample with 0.1 N Na2S2O3 until it turns into yellow, then

adds 1% starch titration continuously until the blue color disappears. Create titration blank using water **instead of a sample**. Reducing sugar levels are calculated by the formula:

Reducing sugar (%): (AT x Fp) / (sample weight x 1000) × 100%

AT = weight of reducing sugar; Fp = dilution factor

Data analysis in this study used a Completely Randomized Design, while quantitative data from each parameter were analyzed using Variance Analysis with a confidence level of 95%. If there is a real difference, then continue with the Duncan Multiple Range Test at a 95% confidence level.

RESULT AND DISCUSSION

Fusarium wilt is a very important and economically harmful disease because until now, there has been no effective chemical control [9]. This disease is caused by the fungus *Fusariumoxysporum* 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 plants. Therefore, Fusarium wilt disease is difficult to control. That is why biological control is very efficient in controlling this disease. Fuction using fusaric acid is one of the biological control methods used to control Fusarium wilt.

Characterization of the cassava planlet has a relationship with the resistance of Fusariumoxysporum. It can be seen from the content of the total reducing sugar content of cassava plantlets. The average reducing sugar levels in Cassava mutants that are resistant to fusarium wilt are shown in Table 1.

TABLE 1. Average Content of Cassava Planlet Reduction Sugar (%)

Treatment (ppm)	Average content of Reducing Sugar (%)
0 (Kontrol)	$3,52 \pm 0,05^{c}$
60	$4,05 \pm 0,03^{bc}$
80	$4,71 \pm 0,29^{b}$
100	$6,14 \pm 0,09^{a}$
120	$6,82 \pm 0,05^{a}$

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

Reducing sugar levels are improved by increasing the concentration of fusaric acid. Reducing sugar level is an increase in cassava plantlets that given fusaric acid treatment and compared with planets without treatment (control). The highest reduction sugar level occurred in the cassava plantlets that were given with fusaric acid scavenging treatment with a concentration of 120 ppm**.** This treatment shows that the higher concentration used in fusaric acid, the higher content of the sugar reduction produced by the cassava plantlet. This can occur because of the influence of fusaric acid on cassava plantlets. The fusaric acid that is induced on cassava plantlets will be absorbed by plants and one of the responses that will arise is the increase in reducing sugar content in cassava plantlets.

According to the study of [10], the amylase and cellulose activity changes because of the lower level of inoculum concentration that impact enough biomass to reduce the formation of products, while the level highest inoculum concentration allows deep produce more biomass, so, the product formation is getting lower. Enzyme hydrolysis is the hydrolysis of starch and cellulose into glucose. Glucose is then used as energy by mold Aspergillusniger for metabolism and growth.

CONCLUSION

Cassava planlets that are resistant to Fusarium wilt disease show different expression characteristics with cassava plantlets that are not resistant to *Fusariumoxysporum*, namely an increase in total dissolved reducing sugar content in cassava planets that are resistant to *Fusariumoxysporum*, along with the increased concentration of fusaric acid.

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