Analysis of Protein Profil of Cassava (*Manihot esculenta* Crantz.) Mutant Plantlets Resistant To Fusarium Wilt.

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ABSTRACT

Cassava (*Manihot esculenta* Crantz.) Is generally grown by Indonesian farmers because it is an important food source of carbohydrates after rice and corn, but there are still many production constraints in cassava cultivation, including Fusarium wilt. This disease is caused by the fungus *Fusarium oxysporum* (*Fo*). Fusarium wilt disease is characterized by the plant wilting rapidly, the roots rot, the plant droops as if it is about to collapse, and white fungal colonies are visible at the base of the tuber. Disease control that does not cause negative impacts can be carried out through in vitro selection in media with the addition of fusaric acid. The purpose of this study was to determine the concentration of fusaric acid that comparing the protein profile between cassava resistant to *Fo* by control. This study used a completely randomized design (CRD) with one factor, namely fusaric acid which was divided into 5 levels of concentration, namely 0 ppm, 60 ppm, 80 ppm, 100 ppm, and 120 ppm. Each of these concentrations was repeated 5 times, and each replication consisted of 2 cassava plantlets (*Manihot esculenta* Crantz.) In each culture bottle. The research data is in the form of qualitative and quantitative data. Qualitative data is presented in descriptive form and supported by photos, while quantitative data from each parameter is analyzed using Analysis of Variance or Anova which is carried out at the 5% real level and further tests with the LSD (Least Significant Difference) test at the real level 5 %.

Key words: Fusaric acid, Cassava (*Manihot esculenta* Crantz.), *Fusarium oxysporum*, *In Vitro*, Protein profile

INTRODUCTION

Cassava (*Manihot esculenta* Crantz.), is the third most important crop in the world and a staple food source and income throughout the tropics (Eleazu *et al*., 2014). Cassava cultivation can be a livelihood for more than 500 million farmers (Amponsah *et al*., 2014). Cassava is an important food commodity in Indonesia, and 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 harvest of food commodities, cassava ranks third after rice and corn, which are the three main sources of carbohydrates in the community (Fauzi *et al*., 2015). One of the problems encountered in cultivating Cassava is Fusarium wilt caused by the fungus *F.oxysporum* (*Fo*) and is medium-transmitted. Arinze (2005); Okigbo (2009) reported that 50% of Cassava tubers produced and harvested in Nigeria were lost due to disease. The main causes of decay of Cassava include: Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae, Collectotrichum spp, Geotrichum candidum, Penicillium chrysogenum, Pennicillium digatum, *Fusarium oxysporum* (Ogunleye *et al*., 2014; Okigbo *et al*., 2015; Gwa *et al*., 2015). This organism reduces the quantity and quality of the plant tubers (Amusa *et al*., 2003). 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 Province reached 8.33 million tons and in 2015 it was 7.39 million tons. This situation makes Lampung a supplier of one-third of national Cassava production from national production of 23.92 million tons (Amonymous, 2015), however there are still many production constraints in Cassava cultivation, including Fusarium wilt disease. This disease is caused by the fungus

Fusarium oxysporum (*Fo*) which until now still cannot be effectively treated. One alternative method of controlling disease that is efficient, effective and safe to the environment, among others, uses resistant varieties. The use of high yielding varieties that are resistant to high yielding fo is one important alternative disease control and does not 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 (Nurcahyani *et al*., 2019; Nurcahyani *et al*., 2019; Nurcahyani *et al*., 2020). Research on Cassava Induced Resistance with fusaric acid (FA) has been carried out previously, and found indications of tolerant FA concentration for selection of in vitro resistant planlets. The outcome of the study, in the form of a Cassava mutant with a new protein bands (molecular weight 100 kDa), missing protein bands (60 kDa molecular weight), and proteins whose bands are consistent and thick (25 kDa).

Material and Methods

The tools used in this research are tweezers, scalpel blade, scalpel, pipette tip, micropipette, test tube, test tube rack, microwave, hot plate, ohaus analytical scales, waterbath, petridish, tissue, shaker, label paper, camera. , electrophoresis apparatus, and spectrophotometer. The materials used in this study were cassava plantlets (*Manihot esculenta* Crantz.) Which had been given pure fusaric acid, 70% alcohol and distilled water, solution A, solution B, solution D, and protein molecular weight marker (Page-Ruler Low Range Unstained Protein Ladder) acrylamide / bisacrylamide solution, Tris-HCl buffer, Sodium Dodecyl Sulfate (SDS), electrophoretic buffer solution, Reducing Sample Buffer (RSB) ((Maniatis *et al*., 1982).
The methods used in this study include:

1. Protein profile of cassava (Manihot esculenta Crantz.) Plantlets using the SDS-PAGE method (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis)

2. Extraction of cassava plantlet protein (Manihot esculenta Crantz.)
3. Protein profile analysis of cassava (Manihot esculenta Crantz.) Plantlet using SDS-PAGE method

a. Protein Concentration Measurement
b. Determination of Protein Molecular Weight (BM)

Result and Discussion

In this study, using cassava plantlet (*Manihot esculenta* Crantz.) *In vitro* and treated with various levels of fusaric acid. The fusaric acid used in this study contained five different concentration levels, namely 0 ppm (control), 60 ppm, 80 ppm, 100 ppm, and 120 ppm. The results of the selection of cassava plantlets that have been induced using fusaric acid with various different concentration levels are presented in Figure 1.

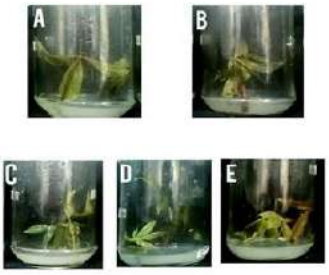

Figure 1. Cassava plantlets with various concentrations of fusaric acid (A) 0 ppm "Control", (B) 60 ppm, (C) 80 ppm, (D) 100 ppm, (E) 120 ppm

From the plantlets, then from each treatment the concentration of fusaric acid was taken as a sample, then analyzed the protein profile.

Protein profile analysis of cassava (Manihot esculenta Crantz.) Plantlet using SDS-PAGE method

Proteins are macromolecules formed from amino acids composed of nitrogen, carbon and oxygen atoms, several types of sulfur-containing amino acids (methionine, cystine, and cysteine) which are linked by peptide bonds. In living things, proteins play a role in forming cell structures and several types of proteins have physiological roles (Bintang, 2010). The results of the protein profile analysis are attached in Figure 2 below.

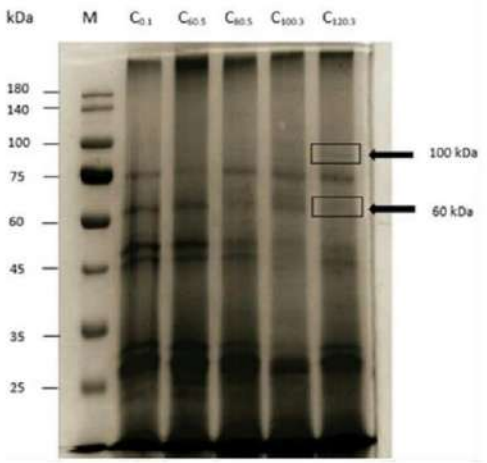

Figure 2. Protein profile of cassava plantlet leaves after induction fusaric acid using the SDS-PAGE method

Based on the results of SDS-PAGE, the protein profile of cassava (Manihot esculenta Crantz.), There were findings in the form of a new band, namely a band at a molecular weight of 100 kDa (fusaric acid concentration treatment 120 ppm), and there was also a missing protein band at a molecular weight of 60 kDa. (Fusaric acid concentration treatment 120 ppm). According to Gunanti *et al*. (2010), the protein band thickness of the SDS-PAGE results illustrates the high and low concentration of a protein contained in the test sample. In this study, the thickest and clearest and also consistent protein bands appeared in almost every individual from each treatment group, namely bands with a molecular weight of 25 kDa. The presence and thickness of the protein bands that are formed depend on the type, number, and sequence of amino acids. This is what causes the differences in each protein that is formed. Likewise, the new band that is formed is the result of a reaction or biochemical process that is formed between plants with the application of both liquid extract and inorganic fertilizers (Salisbury and Ross, 1995a), where in this study the formation of new bands is the result of reactions or biochemical processes. which was formed between cassava and fusaric acid (AF) with different treatments in it.

Conclusion

There are new protein bands (molecular weight 100 kDa) at 120 ppm fusaric acid concentration, missing protein bands (60 kDa molecular weight) at 120 ppm fusaric acid concentrations, and proteins whose bands are consistent and thick (25 kDa) at all concentrations.

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