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# IN VITRO STUDY: INDUCED RESISTANCE OF CASSAVA *(MANIHOT ESCULENTA* CRANTZ.*)* PLANTLET AGAINST *FUSARIUM OXYSPORUM* BASED ON ANALYSIS OF PHENOL CONTENT

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## ABSTRACT

Cassava (*Manihot esculenta* Crantz.) is a tropical and subtropical annual shrub from the Euphorbiaceae tribe. Tuber is widely known as a staple food-producing carbohydrates and leaves as a vegetable. Indonesia is the second largest country producing Cassava after Nigeria with an average of five years' total supply of 9.67 million tons or 10.61% of the total supply of world cassava. Central Cassava land in Indonesia is controlled by Lampung Province with cassava production in Lampung Province reaching 8.33 million tons in 2013. This situation makes Lampung a supplier of one-third of the national Cassava production from national production of 23.92 million tons. However, there are still many production constraints in Cassava cultivation, including Fusarium wilt. This disease is caused by the *Fusarium oxysporum* fungus, which until now still cannot be treated effectively. The use of Cassava cultivars that are resistant to fusarium wilt with high yields is expected to be an important disease control alternative. This study aims to determine and determine the specific expression character of Fo-resistant Cassava plantlets, which are phenol content. The Cassava Planlet has been initiated and selected in vitro in the Murashige and Skoog (MS) medium with the addition of fusaric acid (FA) at a concentration of 0 ppm (control), 20 ppm, 40 ppm, 60 ppm and 80 ppm. The purpose of this study was to determine the phenol content after being induced by fusaric acid and inoculated by *Fusarium oxysporum*. The results showed that in vitro, the concentration range of fusaric acid tolerant for selection of Cassava plantlets with optimum growth was between 20 ppm - 80 ppm; the higher the concentration of FA, the higher the phenol content of the Cassava plantlets.

**KEYWORDS:** Cassava plantlet; *Fusarium oxysporum*; Induced Resistance; in vitro; the phenol content.

## I. INTRODUCTION

Indonesia is the second largest country producing Cassava after Nigeria with an average of five years' total supply of 9.67 million tons or 10.61% of the total supply of world cassava, followed by Brazil, India and United Republic of Tanzania respectively ranged from 8.67 - 4.96 million tons or 9.52% - 5.44%, the rest contributed under 5.30%.[1]

One of the problems encountered in cultivating Cassava is Fusarium wilt caused by the fungus *F.oxysporum* (*Fo*) and is medium-transmitted.[2,3] 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*.[4,5,6] This organism reduces the quantity and quality of the plant tubers.[7]

Disease control on Cassava has been carried out using pesticides which often cause pollution to the environment. Chemicals have been shown to help control Cassava disease, especially when the tuber has been attacked by pathogens.[8] The main problem with chemicals is that it is a big challenge for ecosystems and also the use of chemicals that often predispose them to resistance. One way to control diseases that are safe for the environment is to use varieties that are resistant to induced resistance.[9] The use of superior varieties that are resistant to *Fo* with high yield power is one of the important disease control alternatives and does not cause negative impacts such as the use of pesticides. The development of the *Fo*-resistant Cassava cultivar can be carried out, among others, by the in vitro selection method that is culturing explants in the form of tissue or organs in a medium containing selective concentration fusaric acid.[10]

Fusaric acid (FA) is a metabolite produced by several fungal species from the genus Fusarium. Chemically FA is called 5-n-butylpicolinic acid. This acid can be toxic so that it inhibits growth and culture regeneration,[11,10] but at non-toxic concentrations it helps to induce the synthesis of phytoalexin, a form of plant response to inhibit pathogenic activity.[10] Some parameters can illustrate the occurrence of plant resistance mechanisms against pathogenic infections such as an increase in phenol compounds, an increase in peroxidase enzymes (including PR-protein groups), and the presence of lignification.[12,13,14] Identification of insensitive mutants or variants of FA with in vitro selection was carried out on tomato plants,[15] bananas,[16,17] gladiolus,[18] pineapple,[19] *Sphatoglottis plicata*[20,21] as well as vanilla.[22,23,24,25] The results of the researchers' research showed that somaclonal regeneration of cell mass that is resistant to the toxin was also resistant to pathogens, and this trait was passed on to the next generation. The use of FA in a tolerant concentration so far has never been reported with certainty and precisely in the Induced Resistance Cassava plantlets against *Fo*. Therefore, research on the role of FA as an in vitro inducing resistance is interesting to do.

## II. MATERIAL AND METHODS

Materials research in the form of *Cassava* obtained from previous research. For resistance analysis and phenol content used 70% alcohol, Potato Dextrose Agar (PDA), gallic acid, Folin-Ciocalteau reagent, $Na_2CO_3$, sodium carbonate, 80% ethanol.

Analysis of total phenol compounds using the Singleton & Rossi method.[26] Preparation of standard calibration curve for gallic acid phenol is used as a standard solution. Preparation of sample plantlets prepared according to the method of.[27] Measurement of total phenol was carried out by taking a supernatant of 0.5 mL into a 100 mL volumetric flask, plus 250 µL of Folin-Ciocalteu reagent, after leaving it for 5 minutes then 1 mL $Na_2CO_3$ was added. After mixing evenly, it was put into cuvettes with a volume of 5 mL and observed absorption values at a wavelength of 765 nm using a spectrophotometer (*Beckman DU-65*), distilled water was used as a control. Based on the absorption value then determined the content of phenol compounds based on the gallic acid regression equation, namely the relationship between the absorption value of plantlet extract and the series of gallic acid concentrations.

Qualitative information as the result on this research was consisted as narratives descriptive and supported by photographs. After that, data's were statistically analyzed by Completely Randomized Design. As quantitatively data's from each parameter measured, were compiled and statistically analyzed by analysis of variance (ANOVAs). If the result showed a significantly different, then was continued analyzed by using Duncan Multiple Range Test (DMRT) analysis with accuracy 95%.[28]

## III. RESULTS AND DISCUSSION

Phenol compounds are the result of plant metabolism that is formed with one of the functions as a system of chemical resistance of plants that can prevent the growth and development of pathogens.[12] Before observing the analysis of total phenol compounds in Cassava plantlets for each treatment, a standard curve measurement was performed using gallic acid first. The measurement of this curve is done to estimate the quantity of total phenol with linear regression.[26] From the measurement of this standard curve produced a linear regression line equation (y = 0.002x-0.005) and has a high positive correlation value ($R_2$ = 0.996). This shows that the diversity is homogeneous between the concentration of gallic acid and absorbance. Based on the standard gallic acid curve, the total phenol content of each treatment can be searched based on the regression line equation (Table 1).

**Table 1: Total phenol content (%) of control and fusaric acid (0, 20, 40, 60, and 80 ppm).**

| Treatment (ppm) | Average total phenol content (%) |
|---|---|
| 0 ppm | $5{,}41 \pm 0{,}03^a$ |
| 20 ppm | $6{,}35 \pm 0{,}02^b$ |
| 40 ppm | $6{,}54 \pm 0{,}03^b$ |
| 60 ppm | $6{,}88 \pm 0{,}03^c$ |
| 80 ppm | $6, 92 \pm 0{,}02^c$ |

**Description**: The numbers followed by unequal letters on one column are significantly different based on the Duncan test at 95% confidence degree, after being transformed to √x + Based on Table 1, it is clear that the increase in total phenol levels from about 5.41% in controls, increased to 6.35% at FA stress 20 ppm, followed by 6.54% at 40 ppm, 6.88 ppm at 60 ppm and 6, 92% at 80 ppm. This proves that due to the higher concentration of FA stress, the total phenol content produced will also increase. The increase in the total phenol compound in the Cassava plantlets induced by FA, is another proof of the increase in plant resistance in resisting the rate of *Fo* infection. This is in accordance with the opinion of,[12] which states that one of the parameters for increasing plant resistance to pathogens is the increase in phenol compounds. According to,[10] FA on non-toxic concentrations ($10^{-7}$) stimulates the formation of $H_2O_2$ which is strongly associated with the peroxidase enzyme. Furthermore, this enzyme will oxidize phenol compounds.

The results of this study are similar to the results of a study conducted by,[29] patchouli plants infected by endophytic bacteria and nematodes showed that there was an indication of an increase in phenolic content by *Achromobacter xylosoxidans*. Total phenol levels were also reported by[30] on Chickpea plants infected with *Fusarium oxysporum* f.sp. *ciceris*. From the study it was found that there was an increase in total phenol of around 16-17%.

## IV. CONCLUSION

Based on the results and discussions that have been described in advance can be concluded as follows. The concentration range of fusaric acid tolerant for selection of cassava plantlets with optimum growth is between 20 ppm - 80 ppm. The higher the concentration of fusaric acid, the higher the total phenol level, the Cassava plantlet with *Fusarium oxysporum*.

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