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# Analysis of Cassava (*Manihot esculenta* Crantz) DNA Patterns and Peroxidase Enzyme Activity Results of Induced Resistance with Salicylic Acid

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## Abstract

Cassava (*Manihot esculenta* Crantz) is a food commodity that is ranked third after rice and corn, all three are the main sources of carbohydrates and in the future this commodity will have an increasingly strategic role in people's lives and the country's economy. The growth of cassava plants cannot be separated from diseases caused by pathogens, one of which is a fungus which, if it infects the plant, will multiply and spread so that the plant will be damaged. Biological disease control can be done by using superior varieties, one of which is by administering salicylic acid. The aim of this research was to analyze DNA patterns and determine the peroxidase enzyme activity of cassava plants exposed to salicylic acid compared to controls. This research used a Completely Randomized Design (CRD) with one factor, namely the concentration of salicylic acid which was divided into 5 levels, namely 0 ppm, 80 ppm, 100 ppm, 120 ppm, and 140 ppm with 5 replications each. This research data is presented in comparative descriptive form supported by photos and quantitative data tabulated with different concentrations. The research results showed (1) There was a new (specific) DNA band with a size of 1,100 bp (OPB_14), (2) At a salicylic acid concentration of 100 ppm, the highest peroxidase enzyme activity was 0.193 U/mg/minute.

**Keywords:** Cassava; Salicylic Acid; Induce Resistance; DNA Patterns; Peroxidase Enzyme Activity

## 1. Introduction

Cassava (*Manihot esculenta* Crantz) is a food commodity that is in third place after rice and corn, all three of which are used as the main source of carbohydrates and in the future this commodity will play an increasingly strategic role in people's lives and the country's economy [1]. Lampung Province is one of the provinces which is the largest cassava production center in Indonesia [2]. Based on this statement, cassava production in Indonesia is controlled by Lampung Province and makes Lampung a supplier of one third of national production [3]. Cassava productivity in Lampung province experienced a decline from 2015 to 2019 [4]. A decrease in production yields can be influenced by various factors, both biotic and abiotic, which can inhibit plant productivity [5].

The growth of cassava cannot be separated from diseases caused by pathogens, one of which is fungus, which if it infects the plant will multiply and spread so that the plant will be damaged [6]. Biological disease control can be done using superior varieties, one of which is by applying salicylic acid. Salicylic acid is a signal transduction agent controlling pathogens. Salicylic acid biosynthesis will increase when plants are infected with disease, so that the salicylic acid

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transduction pathway is activated which causes plant resistance to increase. Salicylic acid functions as a phytohormone to stimulate plant tissue growth and increase plant resistance through a systemic resistance system [7].

Salicylic acid applied exogenously can influence biochemical, physiological and molecular processes in plants as an antioxidant [8]. The structure of genes can undergo changes due to mutagens that cause changes in DNA bases. Gene changes will activate defense genes so that mutants that are resistant to a disease will be formed [9], one of which is the gene encoding the peroxidase enzyme. This gene is the dominant hypersensitive resistance gene expressed in plants. This enzyme plays a role in the mechanism of disease resistance so that its activity can be used as a resistance inducer [10]. The use of salicylic acid in tolerant concentrations has so far not been reported with certainty in cassava induced resistance in vivo. Therefore, the aim of this research is to analyze DNA patterns and determine the peroxidase enzyme activity of Cassava plants as a result of salicylic acid exposure and compare it with controls.

## 2. Material and methods

### 2.1. Tools and Materials

The tools and materials used in this research were cassava seeds, autoclave, UV-VIS spectrophotometer, Eppendorff tube, cuvette, centrifuge, PCR machine, salicylic acid crystals, extraction buffer, chloroform: isoamyl alcohol (24:1), 70% ethanol , isopropanol, TBE buffer, primer OPB_14, PCR Premix, Bio line kit 12.5 µL, primer 2.0 µL at a concentration of 100 µM, DNA template 2.0 µL at a concentration of 40 ng/ µL and Nuclease Free Water 8.5 µL , so the total volume is 25.00 µL. Electrophoresis with buffer (500 mL 1x buffer, 1.5% (g/v) agarose minigel, good view), pyrogallol 1.5 mL 0.05 M, and $H_2O_2$ 1% 0.6 mL. This research used a Completely Randomized Design (CRD) with one factor, namely the addition of salicylic acid which was divided into 5 concentration levels, namely 0 ppm, 80 ppm, 100 ppm, 120 ppm, and 140 ppm. Each concentration was repeated 5 times.

### 2.2. Preparation and Planting Cassava

The media used in this research is soil media mixed with husks and processed with compost. In making this planting medium, soil weighing 5 kg/sack contains 4 sacks of husk plus a mixture of organic fertilizer in a ratio of 1:1, then the ready media is put into 25 polybags. Cassava stems are planted after putting the medium in a polybag, then watered in the morning with 100 mL of water. Watering is done twice a week for 30 days to observe growth and development.

### 2.3. Preparation of Salicylic Acid

The salicylic acid used in this research was 5 concentration levels, namely 0 ppm as control, 80 ppm, 100 ppm, 120 ppm and 140 ppm. Making a salicylic acid solution is done by weighing 0.04 g of salicylic acid crystals and putting them in a 500 mL volumetric flask then dissolving them with distilled water until the mark, then homogenizing and obtaining a solution with a concentration of 80 ppm, then for concentrations of 100, 120 and 140 ppm taken respectively as 0.05; 0.06; and 0.07 g salicylic acid. The salicylic acid solution is added and stored in the refrigerator for 1 day. Salicylic acid treatment was carried out when the plants were 14 days old. The addition of salicylic acid to cassava plants is done by pouring 50 ml of salicylic acid solution on the surface of the stem of each cassava plant with various concentrations, namely 0 ppm, 100 ppm, 120 ppm and 140 ppm. Each concentration consists of 5 plants, so each concentration requires 250 ml of salicylic acid solution.

### 2.4. DNA Isolation

DNA extraction was carried out based on the modified Doyle and Doyle method [11], samples of fresh cassava leaves were cut into small pieces then the leaves were weighed at 0.1 gram with an analytical balance. The leaf samples were then crushed using a mortar and pastle, added with 1.5 ml of extraction buffer (CTAB 2%, 100 mM Tris-HCl pH 8, NaCl 1.4 M, 2 mercaptoethanol, 20 mM EDTA pH 8) and put into a tube eppendorf.

The suspension was incubated at 65°C for 40 minutes in a water bath and turned continuously, then centrifuged at 14,000 rpm for 10 minutes. Supernatant was transferred into a new eppendorf tube, and 1x mixture of chloroform: isoamylalcohol (24:1) was added. The eppendorf tube was vortexed until homogeneous and centrifuged at 14,000 rpm for 10 minutes. Then take the top layer (supernatant), and add 0.6x the volume of cold isopropanol (-20°C). The mixture was incubated at -20°C for 1 hour, then the tube was centrifuged at 14,000 rpm for 4 minutes and the supernatant was discarded. The DNA pellet formed at the bottom of the eppendorf tube was washed with 500 µl of 70% ethanol then centrifuged at 14,000 rpm for 3 minutes. The 70% ethanol liquid was discarded and the resulting DNA pellet was dried in air, then 50 µl of TE buffer was added to the DNA pellet until it dissolved and stored at -20 °C.

### 2.5. Analysis of Cassava Plantlet DNA Patterns Using the RAPD Method

PCR analysis was performed according to the method of William *et al.* [12], DNA was prepared as a template that had been dissolved in TE, ice box, and primer OPB_14. The PCR premix was made with the composition (12.5 µL Bio Line kit, 2.0 µL primer at a concentration of 100 µM, 2.0 µL template DNA at a concentration temperature of 40 ng/µL, and 8.5 µL Nuclease Free Water so that the total volume was 25 .00 µL, the premix was amplified using a PCR machine (GeneAmp 2400).

### 2.6. Elektrophoresis

Electrophoresis was carried out using the method of Sambrook et al. [13] in making 500 mL of TBE1x buffer by taking 50 mL of TBE10x buffer solution, then diluting it in a 500 mL measuring cup by adding distilled water to the 500 mL mark and then homogenizing. Agarose minigel 1.5% (g/v) was made by placing 1.5 g of agarose in an Erlenmeyer and adding 100 mL of TBE1x then homogenizing it, then heating using a microwave (t= 100 ºC; ± 2 minutes) until everything was dissolved. The solution was then cooled to a temperature of approximately 50-55 ºC, then 5 µL of Good View was added. A DNA sample of 25 µL (result of running PCR) was inserted into the well in the gel using a micropipette. A total of 10 µL of marker DNA was then inserted into the well at the left end of the gel. The running process is carried out at a voltage of 100 volts for approximately 30 minutes. The electrophoresis results were visualized with UV light from the UV Transilluminator.

### 2.7. Analysis of Peroxidase Enzyme Activity

Peroxidase enzyme activity was analyzed using the method of Saravanan [10]. Cassava leaves were cut finely and weighed 0.5 g then ground with a mortar and pastel then dissolved using distilled water and filtered with filter paper, then added 1.5 mL of 0.05 M pyrogallol, 0.5 mL of 1% $H_2O_2$. The mixture was centrifuged at 5000 rpm for 10 minutes then allowed to stand at room temperature and placed in a 0.5 mL cuvette. Then 100 µl of 1% $H_2O_2$ was added to the cuvette containing the sample mixture and measured on a spectrophotometer with a wavelength of 420 nm using distilled water solution as a blank. Peroxidase enzyme activity was calculated in enzyme units (U) with the calculation 1U = 1 µmol-1.

## 3. Results

### 3.1. Cassava DNA Pattern Analysis using RAPD-PCR

Based on the results of DNA amplification of cassava plants using primer OPB_14 on cassava that was not affected (control) and cassava that was stimulated by salicylic acid with concentrations of 80 ppm, 100 ppm, 120 ppm, 140 ppm, it produced 1 new DNA band. Visualization of the new DNA band patterns obtained from the results of this research is presented in (Figure 1).

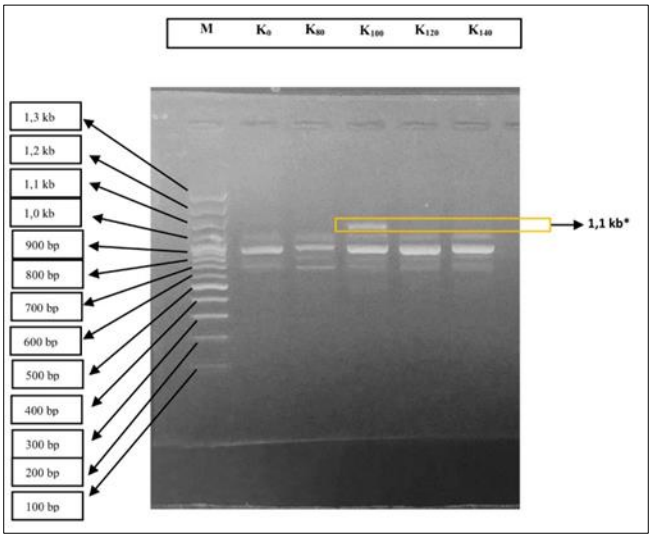


**Figure 1** Cassava DNA banding pattern with primer OPB_14

### 3.2. Analysis of Peroxidase Enzyme Activity

**Table 2** Results of the average honestly significant difference (BNJ) peroxidase enzyme activity of cassava plants after 2 weeks of treatment salicylic acid

| Salicylic Acid Concentration (ppm) | Peroxsidase Enzyme Activity ẏ ± SE (U/mg/min) |
|---|---|
| 0 ppm | $0.087 \pm 0.0008^{d}$ |
| 80 ppm | $0.148 \pm 0.0005^{c}$ |
| 100 ppm | $0.193 \pm 0.0008^{a}$ |
| 120 ppm | $0.164 \pm 0.0012^{b}$ |
| 140 ppm | $0.151 \pm 0.0020^{b}$ |

**Note:** ẏ = Average, SE = Standard Error;Values followed by the same letter are not significantly different at the 5% level BNJ (0.05) = 0.05

## 4. Discussion

The use of RAPD as a molecular marker has been widely used to detect mutational changes in plants. The specific DNA bands produced can be used as characters to group and separate cassava without exposure to salicylic acid (control) from cassava resulting from exposure to salicylic acid. DNA bands that appear in both control and treated plants are considered monomorphism, while DNA bands that only appear in treated plants but do not appear in control plants are considered polymorphism. These differences in DNA band patterns are influenced by differences in genomic DNA amplification. A DNA sample that produces a band indicates that the DNA has a sequence that is complementary to the primer. Polymorphisms produced with RAPD in markers occur due to changes in nucleotide bases, namely deletions or insertions in the genome sequence of leaf samples. Each sample will produce a number of different DNA bands between primers, this is due to differences in primers and DNA sequences [14].

Using primer OPB_14 produced 5 RAPD bands, with 4 monomorphic bands and 1 polymorphic band. Based on (Figure 1), DNA bands measuring 700 bp, 800 bp, 900 bp, 1000 bp were formed in all samples, both control cassava plant samples and cassava resulting from exposure to salicylic acid and there were new (specific) DNA bands in the samples cassava resulting from exposure to salicylic acid with a size of 1,100 bp.

The results of this study are supported by research [1] on DNA analysis of cassava plantlets resulting from exposure to fusaric acid against *Fusarium oxysporum* using the RAPD method, there were specific new DNA bands with sizes of 550 bp (OPA_1) and 300 bp (OPA_10) which were considered as candidate markers RAPD which is resistant to *Fusarium oxysporum*. This specific DNA band can be used as a character to group and separate cassava plantlets without exposure to fusaric acid (control) and cassava plantlets resulting from exposure.

Based on Table 2. The average peroxidase enzyme activity in cassava plants after 2 weeks of salicylic acid treatment shows results that fluctuate according to the concentration of salicylic acid given. The application of 100 ppm salicylic acid is thought to be effective and increases the activity of the peroxidase enzyme as a form of cassava plant sensitivity to defend itself.

According to [10] the gene that regulates the activity of the peroxidase enzyme is the dominant hypersensitive resistance gene in plants, thereby helping the mechanism of resistance to disease. This enzyme plays a role in the mechanism of resistance to disease so that its activity can be used as an induction of resistance. The structure of genes undergoes changes due to mutagens which cause changes in DNA bases [15]. Gene changes will activate defense genes so that mutants that are resistant to a disease will be formed [9]. The resistance mechanism through the salicylic acid pathway is related to proteins related to pathogenesis such as chitinase, peroxidase, β-glucanase and PR-1 which are also associated with changes in gene structure and plant DNA bases. Exogenous administration of salicylic acid can increase the production of secondary metabolites and protect DNA from oxidative damage, thereby giving rise to defense genes, one of which is the gene encoding the peroxidase enzyme [16].

The results of this study are supported by research [17], that there was an increase in peroxidase enzyme activity in vanilla plantlets treated with fusaric acid. The significant increase in peroxidase enzyme activity in vanilla plantlets is thought to be due to treatment with fusaric acid which can trigger an increase in peroxidase compounds ($H_2O_2$). The peroxidase enzyme increases along with the concentration of fusaric acid used in ground orchid plants [18].

The peroxidase enzyme is a protein that is used as plant defense in responding to inducing compounds and also acts as a catalyst in the process of lignin formation and is toxic to pathogens [19]. The peroxidase enzyme is one of the enzymes that play a role in the process of plant resistance to pathogens [20]. Peroxidase functions in resistance through the production of hydrogen peroxide. Hydrogen peroxide can be directly toxic to microorganisms and can also play a role in strengthening cell walls by forming lignin precursors through the activity of the peroxidase enzyme [21].

## 5. Conclusion

The results of the research showed that there were differences in the DNA patterns of control cassava and cassava resulting from salicylic acid exposure, which was indicated by the presence of a new (specific) band with a size of 1100 bp (OPB_14). A salicylic acid concentration of 100 ppm produces the highest peroxidase enzyme activity, namely 0.193 U/mg/minute.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

All authors have no conflicts of interest.

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