Induce resistance of rice plants against bacterial leaf blight by using salicylic acid application

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Abstract. Bacterial leaf blight (BLB) is a disease that can cause yield loss in rice plants. Control efforts can be carried out by increasing the resistance of plants to pathogenic infections by using elicitors in the form of salicylic acid compounds. This study aimed to determine the effect of salicylic acid application to the rice resistance against BLB disease. The research was carried out experimentally in planta in a screen house. The study consisted of pathogen inoculation and salicylic acid treatment with various concentrations (5, 10, 15 mM). The variables observed were pathogenicity, biochemical plant resistance, structural plant resistance, and plant growth. The results showed that there was a suppression of BLB disease intensity in rice infected by X. oryzae due to salicylic acid application. Exogenous salicylic acid correlated with the biochemical resistance increased, especially tannins and phenols, and structural resistance in the form of epidermal thickness and the number of stomata. However, salicylic acid does not affect the growth of rice plants.

1. Introduction
Bacterial leaf blight (BLB) is an important disease in rice plants. The disease is caused by Xanthomonas oryzae. It resulted in yield losses since it can inhibit plant growth. It is informed that this pathogen causes a decrease in yield up to 31% [1]. BLB bacteria infected the rice plants in all growth stages of rice plants, both in the vegetative phase and during the generative phase. In the vegetative phase, the symptoms due to X. oryzae infection are called kresek, while in the generative phase it is called blight which in turn can disrupt the grain filling process [2].

Research to determine the appropriate type of control against BLB pathogens has been carried out [3]. The control using synthetic pesticides is mostly practiced at the farmer's level since it easy to be conducted by farmers and the results can be seen faster. However, it harms the environment, including environmental pollution and residues on plant products.

The control of X. oryzae by inducing resistance is generally carried out by using useful microbes. The use of endophytic bacteria isolated from the roots, stems, and leaves of rice plants can be used to increase rice plant resistance [4]. The induction of plant resistance can also be arranged by chemical compounds application. [5] reported that salicylic acid can be used as an inducer for rice resistance. Research conducted by [6] states, the secondary metabolites in the form of salicylic acid act as a response of plant resistance to pathogens. Based on the previous research, a study was conducted to determine the induce resistance of rice plants against bacterial leaf blight using salicylic acid.
2. Materials and methods

The research was conducted at the screen house of the Faculty of Agriculture, Jenderal Soedirman University, Purwokerto, Central Java. Isolation of bacteria and biochemical analysis was carried out at the Plant Protection Lab and Soil Lab, Faculty of Agriculture, Jenderal Soedirman University.

The materials used in the study included rice seeds IR64, X. oryzae, and salicylic acid. The tools used in the study were rulers, hand sprayers, scales, polybags, glassware, beaker glass, Petri dishes, Bunsen, hemocytometer, spectrophotometer, camera, and stationery.

The study used a randomized block design. The treatments tested were salicylic acid concentrations, consisted of 0mM (M0), 5mM (M1), 10mM (M2), and 15mM (M3) with 6 replications. Stock solution of salicylic acid was made by dissolved salicylic acid in 70% ethanol for 200 mL stock solution. Then, it was sprayed on 40 days old rice plants with various concentrations of 20mL each plant. Inoculation of X. oryzae was carried out by using the clipping method on the 43 days after planting rice plants with concentration 107 cfu mL\(^{-1}\). The tips of the leaves were cut at 5 cm and dipped in the X. oryzae suspension for 5 minutes. The plants were covered with transparent plastic for 3-4 days to maintain the moisture.

The variables observed were disease intensity, AUDPC, biochemical resistance in the form of quantitative tannin, saponin content, and qualitative phenol content, structural resistance in the form of epidermal thickness, and stomata. The disease intensity was calculated with formulas as follow:

\[
DI = \frac{a}{b} \times 100\% 
\]

Note: DI= disease intensity, a = the leaf length with symptom (cm), b = the length of leaf (cm). The total area under the disease progression curve (AUDPC) was calculated using the formula as follow:

\[
AUDPC = \frac{\sum_{i=1}^{n} \left( Y_i + Y_{i+1} + 1 \right)}{2} \left( t_i + 1 - t_i \right) 
\]

Note: AUDPC = Disease progression curve, \( Y_i + 1 = \) Observation data \( i + 1 \), \( Y_i = \) First observation data, \( t_i + 1 = \) Time of observation \( i + 1 \), \( t_i = \) Time of first observation.

The saponin test was conducted by weighing 1 g of plant extract, and then added with warm water, shaken vertically for 10 seconds, and left for 10 seconds. Foam as high as 1–10 cm that is formed and stable for no less than 10 minutes indicates the presence of saponins. The addition of 1 drop of 2 N HCl will increase the stability of the foam.

The tannin test was arranged by pounding 5 g of the sample using a mortar, then 80% ethanol was added, then filtered. A total of 20 mL of filtered filtrate was added 3 drops of 10% FeCl3. The formation of a dark blue or greenish-black color indicates the presence of tannins. The analysis of total phenol was performed using a spectrophotometer method with the addition of Follin Ciocalteu solution. Gallic acid is used as a standard in various concentrations to form a colorimetric reaction which will be used as a standard curve. The wavelength required for phenol analysis is 765 nm.

The thickness of the epidermis of rice leaves was observed by washing the leaves in running water, fixed with FAA for 24 hours, and rinsed with distilled water. Then the leaves were cut thinly using a razor blade crosswise, rinsed with 5% NaOCl, rinsed with distilled water, add by 0.25% safranin dye, then the leaf slices were placed in a glass preparation that has been given 30% glycerin. Observations were made using a light microscope with a magnification of 40 x to observe the epidermis.

Leaf tissue observed in the cross-section of the leaf includes the thickness of the cuticle and adaxial and abaxial epidermis. The calculation was carried out with the following formula:
Note: TK (thickness of cuticle and epidermis), TKM (thickness of cuticle and epidermis at micrometer), and K (calibration)

The method to measure the density of the stomata was using a replica method. The top and bottom surfaces of the rice leaf samples were cleaned using a tissue to remove dirt. Smeared with clear nail dye, and left for 5 minutes to dry. The dry spread was covered with clear tape and flattened. The tape peeled off, then sticks it on the glass object, flattens it, and labelled. Microscope with a magnification of 10x10 was used for observation of the number of stomata. The observed variable was the number of stomata for each field of view calculated using the following formula:

\[
Stomata\ density = \frac{The\ number\ of\ stomata}{The\ unit\ area\ of\ view}
\]

3. Results and discussion
The results of observations for pathogenicity parameters, including disease intensity and AUDPC are shown in the following table.

| Treatments  | Disease intensity | AUDPC (% days) |
|-------------|-------------------|---------------|
|             | 1 | 2 | 3 | 4 | 5 |               |
| M0 (control)| 7.50 a | 12.50 a | 23.75 a | 36.25 a | 50.63 a | 0.17         |
| M1 (5mM)    | 5.86 b | 7.86 b | 11.71 b | 11.71 b | 16.00 b | 0.07         |
| M2 (10mM)   | 4.17 b | 5.00 b | 9.17 bc | 10.00 b | 15.83 b | 0.06         |
| M3 (15mM)   | 2.67 c | 3.83 c | 7.50 c | 9.17 b | 13.33 b | 0.05         |

Note: M0=control (without salicylic acid), M1= salicylic acid 5mM, M2= salicylic acid 10mM, M3= salicylic acid 15mM.

Figure 1. Development of BLB disease in rice plants with salicylic acid.
Based on the results, the disease intensity of control experienced higher than the treatment with various concentrations of salicylic acid application, since the first observation (Table 1.). Furthermore, the lowest disease intensity was indicated by treatment with the highest concentration of salicylic acid (15 mM). This phenomenon suggested that the salicylic acid application to rice plants has a function as an elicitor to induce plant resistance. Therefore, it can suppress the disease intensity. The ability of salicylic acid as plant resistant inducer has been observed in several plants. According to [7], salicylic acid induces the resistance of tomato plants against fungal pathogens, *Fusarium oxysporum* f.sp. *Lycopersici*. The study revealed that salicylic acid plays a role in systemic acquired resistance, especially in transmitting the defense signal from the infected part of the plants.

The disease progress graph (Figure 1) shows that the AUDPC values of all treatments with various concentrations of the salicylic acid application were less than the AUDPC value of the control treatment. This suggested the ability of salicylic acid to suppress disease progression. In line with this phenomenon, a study conducted by [8] also showed the ability of salicylic acid to decrease of AUDPC value in hybrid rice infected by several pathogens in the sheath.

### Table 2. Effect of salicylic acid on the biochemical activity parameters.

| Treatments | Tanin | Phenol (ug GAE/10 ml) |
|------------|-------|-----------------------|
| M0 (control) | -     | 15.99                 |
| M1 (5mM)    | +     | 8.18                  |
| M2 (10mM)   | +     | 15.95                 |
| M3 (15mM)   | +     | 18.00                 |

Based on the qualitative data on tannin content, it is known that control plants infected with BLB pathogens without salicylic acid application did not show a blue-black or greenish-black discoloration as characteristic of the presence of tannin content in plant tissue. Meanwhile, the rice leaves treated with salicylic acid at various concentrations showed a change in color from greenish-brown to blackish blue, which indicates the presence of tannins. According to [9], tannins are defined as polyphenolic compounds as antimicrobial compounds which able to inhibit the growth of microbes. Presumably, tannins accumulation in this research is due to the increased plant resistance by salicylic acid as an elicitor.

### Table 3. Effect of salicylic acid on the structural resistance of rice plants infected with *X. oryzae*.

| Treatments | Epidermis thickness | Number of stomata |
|------------|---------------------|-------------------|
| M0 (control) | 16                  | 0.80/µm²          |
| M1 (5mM)   | 14                  | 0.68/µm²          |
| M2 (10mM)  | 14                  | 0.63/µm²          |
| M3 (15mM)  | 21                  | 0.71/µm²          |

The phenol content as a biochemical resistance parameter was observed quantitatively by using a spectrophotometer. According to [10], phenol is a secondary metabolite which is the main compound for the plant's growth and plays an important role in plant defense mechanisms. Based on Table 2, the phenol content increased concomitant with the increase of the salicylic acid concentration applied to plants. However, rice plants sprayed with salicylic acid at concentrations of 5mM and 10mM had lower phenol content than the control. It is indicated that 15mM salicylic acid is the most appropriate concentration to apply to elevate the phenol accumulation in the rice plant. Previously, the accumulation of phenol triggered by exogenous salicylic acid to induce plant defense was observed in chickpea [11].
Plant response to the induced resistance was also determined by structural mechanism as shown in Table 2. The increment of the salicylic acid concentrations affected the increase of leaf epidermis thickness. The increase in epidermal thickness is thought to be related to an increase in the concentration of salicylic acid applied exogenously. Salicylic acid is involved in the process of lignin formation (lignification) in plant tissue [12]. Lignification generally occurs in the epidermis of the plant.

Table 4. The effect of salicylic acid application to plant height of rice plants infected with *X. oryzae*.

| Treatments   | Observation |
|--------------|-------------|
|              | 1 | 2 | 3 | 4 |
| M0 (control) | 34.42 | 57.33 | 77.08 | 81.92 |
| M1 (5mM)     | 35.13 | 58.33 | 79.08 | 81.83 |
| M2 (10mM)    | 31.33 | 56.92 | 73.58 | 79.92 |
| M3 (15mM)    | 33.33 | 58.00 | 77.08 | 82.75 |

Table 5. The effect of salicylic acid application to number of leaves of rice plants infected with *X. oryzae*.

| Treatments | Observation |
|------------|-------------|
|            | 1 | 2 | 3 | 4 |
| M0 (control) | 5.08 | 10.50 | 14.17 | 17.36 |
| M1 (5mM)    | 5.17 | 10.58 | 13.17 | 19.75 |
| M2 (10mM)   | 4.92 | 10.33 | 12.58 | 16.83 |
| M3 (15mM)   | 5.00 | 10.75 | 14.17 | 17.17 |

Table 6. The effect of salicylic acid application to number of tillers of rice plants infected with *X. oryzae*.

| Treatments | Observation |
|------------|-------------|
|            | 1 | 2 | 3 | 4 |
| M0 (control) | 0 | 1.50 | 2.58 | 2.83 |
| M1 (5mM)    | 0 | 1.64 | 3.08 | 3.17 |
| M2 (10mM)   | 0 | 1.67 | 2.67 | 2.92 |
| M3 (15mM)   | 0 | 1.82 | 3.08 | 3.42 |

The number of stomata in the control tended to be more than the plants treated with salicylic acid. This occurrence also shows in the application of 50mg/l salicylic acid in canola [13]. Stomata are one of the natural openings for pathogens to invade the plants. The greater the number of stomata, the easier it is for pathogens to enter the plant tissue. Therefore, the plants with a smaller number of stomata tend to have higher resistance.

The salicylic acid treatments with various concentrations showed no effect on the growth of rice plants. Growth parameters such as plant height, number of leaves, and number of tillers observed during vegetative growth are presented in Table 4, 5, and 6. This occurrence was opposite with research conducted by [14] which stated that salicylic acid can play a role as a plant hormone that is involved in the growth and development of the plants. Presumably, the salicylic acid application in this research is more related to plant resistance compared to plant growth.
4. Conclusion
The salicylic acid applications induce the plant resistance to X. oryzae infection by suppression of plant disease intensity, increasing the response of biochemical, and structural resistance. The best concentration of salicylic acid to induce resistance the rice plant against X. oryzae from the results of the study was 10mM. Salicylic acid does not affect the growth of rice plants.

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