Application of salicylic acid to induce disease resistance against fusarium wilt on banana

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Abstract. Fusarium wilt is the most destructive disease on banana. One of control measures that can be performed to control this disease is by increasing plant immunity. The aim of this study was to find out the disease resistance profile of banana to fusarium wilt through resistance induction with Salicylic acid. This study was performed at Plant Pest and Disease Laboratory, Indonesian Tropical Fruit Research Institute, started from May to December 2018. Banana cultivars used in this study were cv. Barangan and Raja Kinalun at pre-planting stage. The treatments were the induction methods consisted of control (sterile water), Salicylic acid spraying to entire part of plant and the root soaking with 5 mM Salicylic acid solution. The plants were artificially inoculated with F. oxysporum f.sp cubense VCG 01213/16 (TR4). Each treatment was repeated 3 times with 10 plants for each treatment unit. The result showed that banana cv. Barangan inoculated with Foc (control) was highly susceptible while cv. Raja Kinalun in the same treatment had moderate resistant criterion. Salicylic acid can induce resistance to fusarium wilt disease on cv Barangan with moderate resistant criterion compared to highly susceptible on its positive control.

1. Introduction
Fusarium wilt is the main disease that attacks banana plants. This disease causes huge yield losses. The disease is caused by Fusarium oxysporum f. sp cubense (Foc). Various efforts have been made to control this deadly disease. The fact is that there are no effective and efficient control techniques available to overcome fusarium wilt attacks in infected areas [1]. In order to obtain effective control techniques, it is necessary to know the way of life of the pathogens that cause the disease.

Pathogens are divided into 3 groups based on their way of life, namely biotroph, necrotroph and hemibiotroph. F. oxysporum is more precisely grouped into hemibiotroph which have the characteristics of both pathogens, biotrophs and necrotroph groups [2]. As a biotroph, pathogens are controlled by plant defense mechanisms through the Salicylic acid (SA) pathway. Moore et al. [3] grouped F. oxysporum into the necrotroph group which obtained energy from food sources in the form of dead cells, they attacked and killed plant tissues and lived as saprotrophs on the remaining dead tissue. Based on these pathogen types, Foc is controlled by plant defense mechanisms through the Jasmonate and Ethylene pathways.

In plant defense systems against pathogens, there is the terminology of Systemic Aquired Resistance (SAR), which is a systemic defense system (all parts of the plant) due to local infection [4] or after being treated with synthetic or natural compounds [5]. SAR is a plant defense mechanism that...
can be induced and produces immunity against various microorganisms. SAR requires signal molecule SA and is associated with accumulation of pathogenesis related (PR) proteins [6]. When plants are infected with pathogens, SA accumulation will occur which functions as a systemic signal that is transported through phloem tissue to activate plant defense systems [7]. SA is able to induce banana resistance to Foc in the post acclimatization phase if the previous plantlets have been applied with SA on tissue culture media [8]. SA application increases the expression of PR1 and PR3 at 24 hr after treatment as indicated by the increase in the number of mRNAs produced. [9].

One way to increase the resistance of banana plants to fusarium wilt can be obtained by improving plant immunity. Plant immunity can be stimulated with SA and Jasmonic acid. Suryanti et al. [8] stated that the addition of SA to banana tissue culture media can increase the resistance of banana plantlets to fusarium wilt with LC50 (salicylic acid toxicity which causes shoot death 50%) for the use of SA in vitro is 9.8 ppm. The application of exogenous SA delayed the onset of symptoms due to the infection of the MDMV virus in corn plants [10]. Naturally the implementation of SAR is a hypersensitive response (HR), the production of defense enzymes such as PR1, 1,3-β-glucanase (PR2), chitinase (PR3), phenylalanine ammonia-lyase (PAL), peroxidase (POX) and others [11]. The induction of immunity is expected to be inherited to the saplings because banana plants are generally propagated through the separation of the saplings.

Banana cv. Barangan is one of commercial cultivars in Indonesia. However, this cultivar is well known as very susceptible to fusarium wilt while banana cv. Raja Kinalun is more resistant to this disease attack. Therefore, a research had been done to determine the resistant profile of banana cv. Barangan and Raja Kinalun against Fusarium wilt disease after being treated with SA.

2. Methods

2.1. Material

The materials used in this study were: banana plantlets cv. Barangan and Raja Kinalun, salicylic acid (SA), sand, rice husk charcoal, fertilizers and F. oxysporum f. sp. cubense VCG 01213/16.

2.2. Method

2.2.1. Acclimatization of banana plantlets and application of SA. The banana cultivars used in this study were cv. Barangan (susceptible to Foc VCG 01213/16) and cv. Raja Kinalun (relatively resistant to Foc VCG 01213/16). Plantlets were acclimatized in plastic tray for 1 mo in sterile media containing soil: rice husk charcoal (1:2). This treatments, namely SA application by spraying all parts of the plant until wet [9]. SA application by soaking plant roots for 15 min and as a control of plant roots soaked in sterile water for 15 min and all parts of the plant also sprayed with sterile water. SA solution was applied at concentration of 5mM. Foc inoculation was performed by soaking the plant roots into Foc suspension containing 10⁶ conidia/mL for 5 min.

2.2.2. The growth of banana plantlets after SA treatments. The growth of banana plantlets after treated with SA was observed weekly. Plant height, pseudostem diameter and number of leaves were recorded until 2 mo after treatments. At the end of observation period, the corm disease severity index was assessed.

2.2.3. Disease resistance testing after SA applications. The double cups method was used for disease resistance testing (12). Plantlets were planted in a plastic cup containing a mixture of sterilized sand: rice husk charcoal = 1: 1 (v / v). This study was arranged in a randomized block design consisting of 6 treatments which were the combinations of 2 cultivars of banana: 1. Barangan and 2. Raja Kinalun and 3 resistance induction treatments: 1. control (sterile water), 2. SA application by spraying SA to all part of plantlets and 3. SA application by soaking plantlet roots with SA solution. Each treatment replicated 3 times. Each treatment unit consisted of 10 plants. As a negative control, banana plantlets
for control (sterile water spraying), SA spraying or SA root soaking were not inoculated with Foc. The treatments scheme and the number of samples needed were shown in Table 1.

### Table 1. Number of plantlets used for each treatment combination.

| Treatments          | Induction of resistance | Total plantlets |
|---------------------|-------------------------|-----------------|
|                     | Control | SA Spraying | SA Soaking |               |
| 1. Barangan         |         |             |            |               |
| a. Not inoculated   | 10      | 10          | 10         | 30             |
| b. Inoculated       | 30      | 30          | 30         | 90             |
| 2. Raja Kinalun     |         |             |            |               |
| a. Not inoculated   | 10      | 10          | 10         | 30             |
| b. Inoculated       | 30      | 30          | 30         | 90             |

2.2.4. The observed variables. In this study we observed several variables as follow:

1. The incubation period was observed starting a day after the treatment until the initial symptoms of the Foc attack in the form of yellowing on the edge of the old leaf strands.
2. Growth parameters consisted of plant height, stem diameter and number of banana plant leaves after being inoculated by Foc. Growth parameters were observed every wk until the plants showed symptoms of severe disease or until the end of the study (2 mo after inoculation).
3. The percentage of attacked plants, calculated at the end of the observation (2 mo after treatment) using the formula:
   \[
   \text{Percentage of plant attacked} = \frac{T1}{T2} \times 100\%
   \]
   \[
   T1 = \text{Number of affected plants per treatment},
   \]
   \[
   T2 = \text{Number of plants observed}.
   \]
4. The disease severity index in the leaves, calculated the number of yellowing symptomatic leaves on the treated plants. Then damage scoring was carried out based on the modified scale of Mohammed (12),
   \[
   1 = \text{no symptoms in leaves (healthy plants),}
   \]
   \[
   2 = 1-10\% \text{ leaves turn yellow / symptomatic,}
   \]
   \[
   3 = 11-25\% \text{ of leaves turn yellow / symptomatic}
   \]
   \[
   4 = 26-50\% \text{ of leaves turn yellow / symptomatic,}
   \]
   \[
   5 => 50\% \text{ of leaves turn yellow / symptomatic,}
   \]
   \[
   6 = \text{the plants die.}
   \]
   Observations are carried out every week until 2 mo after treatment.
5. The corm disease severity index was carried out when the plant showed symptoms of a severe disease attack (> 50% of the leaves were yellowing / scale 5) or at the end of the observation if the plants did not show external symptoms of disease (until 2 mo after treatment). The corm was cleaned and all the roots was removed, and then cut transversely on the half of the corm. The corm disease severity index was used based on the Jones (1994) in Rahmi et al. [13] scales.
   \[
   1 = \text{no black spots on the corm tissue,}
   \]
   \[
   2 = \text{the black spots cover } \frac{<1}{3} \text{ of the corm tissue,}
   \]
   \[
   3 = \text{the black spots cover } \frac{1}{3} \text{ of the corm tissue,}
   \]
   \[
   4 = \text{the black spots cover the } \frac{1}{3}-\frac{2}{3} \text{ of corm tissue,}
   \]
   \[
   5 = \text{the black spots cover } > \frac{2}{3} \text{ of the corm tissue,}
   \]
   \[
   6 = \text{the black spots cover almost all part of the corm/ the plants die.}
   \]
   The disease severity in leaves on leaves (LDSI) and corm (CDSI) were calculated by the formula:
Plant resistance categories were assessed based on percentage of affected plants and the leaf disease severity index (LDSI) and the corm disease severity index (CDSI) with modified scales made by [12] as stated on Table 2. Parameters for growth and disease attack were observed every week. Data analysis was carried out descriptively.

**Table 2.** The fusarium wilt disease resistance category based on the percentage of affected plants, as well as leaf (LDSI) and corm disease severity index (CDSI).

| Disease percentage (%) | Disease severity indices | Resistance category |
|------------------------|--------------------------|---------------------|
|                        | Leaf (LDSI) | Corm (CDSI)       |                     |
| 0                      | 1            | 1                  | Highly resistant    |
| ≤ 20                   | 1 – 2        | 1 – 2              | Resistant           |
| 20 – 50                | 2 – 3        | 2 – 3              | Moderate resistant  |
| 50 – 75                | 3 – 4        | 3 – 5              | Susceptible         |
| > 75                   | > 4          | > 5                | Highly susceptible  |

3. Results and discussion

3.1. The growth of banana plants after application of SA

Banana plants that were applied to SA before planting showed symptoms of wilting due to SA treatment at the first week after treatment, especially in the treatment of root soaking. However, the plants returned fresh in the next week (Fig. 1).

**Figure 1.** Banana cv. Raja kinalun at 1 week (left) and 2 week after planting (right). Plant treated with SA by root soaking method showed wilt symptom at the first week (c and f) however the plants grew normally after two week (c and f).

Banana cv. Barangan that were applied to SA and inoculated with Foc showed higher plant height, stem diameter and number of leaves at 8 wk after planting compared to plants which were only Foc inoculated without the application of SA. This showed that the plants sprayed with SA were able to grow well even though at the beginning of the treatment the plants looked rather wilted due to the
treatment given. The increasing of plant height, stem diameter and number of leaves of banana cv. Barangan and Raja Kinalun at 8 wk after planting are shown in Table 3.

**Table 3.** The increasing of plant height, pseudostem diameter and number of leaves of banana cv. Barangan and Raja Kinalun at 8 wk after planting.

| Treatments                  | Plant height (cm) | Pseudostem diameter (mm) | Number of leaves (sheet) |
|-----------------------------|-------------------|--------------------------|--------------------------|
| cv. Barangan                |                   |                          |                          |
| Control (Foc -)             | 8.2 ± 2.3         | 9.5 ± 1.0                | 4.8 ± 0.8                |
| SA spraying (Foc -)         | 8.0 ± 2.9         | 8.9 ± 0.9                | 3.8 ± 1.5                |
| SA soaking (Foc -)          | 7.3 ± 1.1         | 6.8 ± 2.3                | 4.8 ± 1.3                |
| Control (Foc +)             | 6.8 ± 0.3         | 8.0 ± 0.8                | 4.6 ± 0.3                |
| SA spraying (Foc +)         | 8.9 ± 0.4         | 9.1 ± 0.1                | 4.9 ± 0.3                |
| SA soaking (Foc +)          | 8.8 ± 1.0         | 9.0 ± 0.0                | 5.1 ± 0.4                |
| cv. Raja Kinalun            |                   |                          |                          |
| Control (Foc -)             | 6.1 ± 0.8         | 6.4 ± 0.8                | 5.3 ± 0.6                |
| SA spraying (Foc -)         | 6.0 ± 1.6         | 6.0 ± 1.4                | 7.0 ± 1.0                |
| SA soaking (Foc -)          | 4.8 ± 0.3         | 5.2 ± 0.5                | 7.0 ± 1.0                |
| Control (Foc +)             | 5.6 ± 1.8         | 5.8 ± 0.9                | 5.4 ± 1.0                |
| SA spraying (Foc +)         | 3.8 ± 1.3         | 5.3 ± 0.8                | 5.3 ± 0.7                |
| SA soaking (Foc +)          | 4.7 ± 1.1         | 6.0 ± 0.6                | 4.7 ± 1.4                |

Plants treated with SA, grew slightly hampered in the first week after planting compared to plants that were not treated with it, but in the following week plant growth looked better even though the plants were inoculated with Foc. Symptoms of abnormal plant growth were seen in some plants treated with SA with the root immersion method. Plants did not grow at the point of growing shoots like banana plants in general but grew on other new shoots while early shoots die or grew far below the previous shoots of the plants (Fig. 2). This condition caused some plants had lower height compared to previous observations. This condition is then followed by the emergence of many leaves but in small size. This incident was found in about 30% of banana cv. Barangan which were treated by soaking the roots with SA while rarely found at treatments of SA by spraying and control. However, it cannot be ascertained whether the incident was caused by the treatment of soaking the roots with SA or caused by other things. The appearance of plants that were partially grew abnormally on the treatment of root immersion with SA shown at Fig. 2.
Figure 2. Abnormal plant growth observed on plants treated with root soaking on 5mM SA for 15 min (a and b), the new shoot (arrow sign) appears on the pseudostem base and then the former shoot dies (b) and the abnormal plant growth (c1) and a normal plant growth appearance (c2).

3.2. The effect of SA application on the development of symptoms of Fusarium wilt disease in banana cv. Barangan and Raja Kinalun

The application of salicylic acid by spraying the entire parts of the plant and soaking the roots can increase the resistance profile of banana cv. Barangan to fusarium wilt. Banana cv. Barangan which was artificially inoculated with Foc VCG 01213/16 without salicylic acid treatment was categorized as highly susceptible while plants sprayed with SA showed increased resistance to a moderate resistant category (Table 4). In line with this result, previous research stated that SA applied to in vitro culture process was also able to increase the resistance of banana plants to fusarium wilt [8]. Plants treated with SA showed delayed symptoms of wilt disease than without SA treatment. Positive control plants were attacked at 2 wk after inoculation while in plants treated with symptomatic SA the disease appeared at 4 wk after inoculation. Banana cv. Raja Kinalun in all treatments did not show symptoms of fusarium wilt until 4 wk after inoculation with Foc VCG 01213/16.

Banana cv. Raja Kinalun was more resistant to fusarium wilt than cv. Barangan. At the end of the observation (8 wk after planting) there were no symptoms of a severe disease attack on cv. Raja Kinalun while many of the Barangan had died caused by disease attacks. Plants that were not inoculated with Foc either those at control plants or those applied to SA did not show tissue necrosis at all for both banana cv. Barangan and Raja Kinalun. Plants that were inoculated with Foc showed necrosis with varying intensity. The most severe disease intensity of the corm was found in banana cv. Barangan, treated with Foc inoculation (positive control), then by soaking root treatment and the mildest symptom found in plants sprayed with a 5 mM SA solution (Fig. 3). While for banana cv. Raja Kinalun, although the external symptoms of the disease were not visible, but there were symptoms of necrosis on the corm. It is likely a resistance mechanism occurs in this cultivar so that the attack on the root can be controlled to inhibit further spread to the pseudostem. Plants that survived after 8 wk of observation were transferred to polybags containing soil and manure to observe the development of symptoms of fusarium wilt disease.
Table 4. Fusarium wilt disease percentage, leaf disease severity index (LDSI), corm disease severity index (CDSI) and resistance category of banana cv. Barangan and Raja kinalun after treated with SA at 8 wk after planting.

| Treatments                  | Disease percentage (%) | LDSI | CDSI | Resistance category  |
|-----------------------------|------------------------|------|------|----------------------|
| cv. Barangan                |                        |      |      |                      |
| Control (Foc -)             | 0.0                    | 1.0  | 1.0  | Highly resistant     |
| SA spraying (Foc -)         | 0.0                    | 1.0  | 1.0  | Highly resistant     |
| SA soaking (Foc -)          | 0.0                    | 1.0  | 1.0  | Highly resistant     |
| Control (Foc +)             | 90.0                   | 4.7  | 5.3  | Highly susceptible   |
| SA spraying (Foc +)         | 47.6                   | 2.6  | 2.7  | Moderate resistant   |
| SA soaking (Foc +)          | 68.6                   | 3.5  | 4.3  | Susceptible          |
| cv. Raja Kinalun            |                        |      |      |                      |
| Control (Foc -)             | 0.0                    | 1.0  | 1.0  | Highly resistant     |
| SA spraying (Foc -)         | 0.0                    | 1.0  | 1.0  | Highly resistant     |
| SA soaking (Foc -)          | 0.0                    | 1.0  | 1.0  | Highly resistant     |
| Control (Foc +)             | 20.0                   | 3.0  | 4.2  | Moderate resistant   |
| SA spraying (Foc +)         | 10.0                   | 2.3  | 2.8  | Resistant            |
| SA soaking (Foc +)          | 10.0                   | 2.9  | 2.1  | Resistant            |

*SA = Salicylic acid.

Figure 3. Fusarium wilt disease symptoms on cv Barangan. A. Negative control (without foc inoculation), B. Foc inoculated control (positive control) and C. SA spraying + Foc inoculation.
4. Conclusion
Banana cv. Raja Kinalun is more resistant to fusarium wilt than cv. Barangan. The application of 5 mM SA by spraying the entire banana plant tissue can increase the resistance of banana cv. Barangan to fusarium wilt about 50%. Observation of the development of disease incidence and disease severity on plants treated with SA needs to be continued in order to obtain more valid result.

References
[1] Ploetz R C 2006 *Phytopathology* **96** 653–6
[2] Agrios G N 2005 *Plant pathology* (New York: Elsevier Academic Press)
[3] Moore D, Robson G D, Trinci A P J 2016 *21st Century Guidebook to Fungi* (Built by David Moore with Course Genie and Dreamweaver)
[4] Vlot A C, Klessig D F, Park S W 2008 *Curr. Opin. Plant. Biol.* **11** 436-442
[5] Lloren E, Garcia-Agustin P, Lapeña L 2017 *Sci. Agric.* **74**(1) 90-100
[6] Durrant W E, Dong X 2004 *Annu. Rev. Phytopathol.* **42** 185-209
[7] Shulaev V J, León J, Raskin I 1995 *Plant Cell.* **7** 1691–1701.
[8] Suryanti, Chinta Y D, Sumardiyono C 2009 *J. Perlindungan Tanam. Indones.* **15**(2) 90–5
[9] Endah R, Beyene G, Kiggundu A, van den Berg N, Schluter U, Kunert K, et al 2008 *Plant Physiol. Biochem.* **46** 1007–1014
[10] Cueto-Ginzo I A, Serrano L, Sin E, Rodriguez R, Morales JG, Medina V, et al.2016 *Physiol. Mol. Plant. Pathol.* **96** 47–59
[11] Heil M 2007 Trade-offs associated with induced resistance In: Induced Resistance for Plant Defence: A Sustainable Approach to Crop Protection. In: Walters D, Newton A, Lyon G, editors. (UK: Blackwell Publishing) pp 157–77
[12] Mohammed A, Mak C, Liew K, Ho Y1999 Early evaluation of banana plants at nursery stage of fusarium wilt tolerance. In: The International Workshop on Banana Fusarium Wilt Diseases. INIBAP 174–85.
[13] Rahmi Anna F, Nasir N, Jumjunidang, Hermanto C 2014 Uji *J. Biol. Univ. Andalas.* 1–8.