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Preface

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Preface

First and foremost, it is our great pleasure to welcome all of our distinguished forum guest and invited speakers, presenters, and participants of the 4th International Conference on Agriculture and Life Sciences 2020 (ICALS 2020). The world are now facing the COVID-19 pandemic that threatens human health and disrupting other aspects of human life. A new life order that called as "New Normal" has been introduced to the community in order avoid COVID-19 infection during their life activities. One of the things that is emphasized in implementing the New Normal is having a healthier lifestyle through the consumption of healthy and nutritious food. The industrial crops are thought to have an important role in the provision of healthy food or health supplements. Therefore, the main theme of this conference is "**Retouching Strategy for Exploring Potency of Industrial Crops for Health in Adapting to the New Normal Era**". Due the implementation of Indonesian government regulations to limit the spread of COVID-19 viral infection through the prohibition of gathering activities with many participants, the current ICALS was held in the virtual format instead to be postponed.

This virtual conference was held on 6 – 8 October 2020 in Faculty of Agriculture University of Jember, Indonesia. It is an ongoing effort by the Faculty of Agriculture University of Jember, starting from 1st ICALS as International Seminar and Workshop of Plant Industry (ISWPI) on 2017, the International Seminar and Workshop of Plant Industry (ISWPI) on 2018, 3rd International Conference on Agriculture and Life Sciences (ICALS 2019) on 2019. The ICALS 2020 is co-organized by Faculty of Agriculture University of Jember, Graduate Program University of Jember, Implementation Programs Unit of Islamic Development Bank University of Jember, and Center of Excellence on Crop Industrial Biotechnology (PUI-PT-BioTIn).

The plenary session of the international seminar presented two keynote speakers from the University of Jember and from the Ministry of Agriculture, Republic of Indonesia with 40 minutes for lecture for each. The guest and invited speakers from South Korea, Japan, Belgium, Germany, and Indonesia have been participated in this conference to share their knowledge and expertise on 30 minutes of presentation. The ICALS 2020 was remotely attended by 1262 participants from academician, researchers, students, farmers, private business, and governments from total 8 countries (Indonesia, Malaysia, India, Australia, Japan, Belgium, Germany, South Korea) and 13 provinces in Indonesia (East Java, Central Java, Jogjakarta, West Java, South Sumatra, North

Sumatra, West Sumatra, South Sulawesi, South East Sulawesi, North Sulawesi, Bali, West Nusa Tenggara, and Papua). Among this number, 160 participants disseminated their scientific result related to this conference topic. This virtual conference was successfully delivered using Zoom application and discussion sessions were conducted by means of the presenters answering questions that were raised through the chat menu.

Last but not least, we would like to express blessed gratitude to University of Jember for their support to success of this virtual conference and also, a heartfelt wish to all the committee involved in ICALS 2020.

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The potency of plant resistance inducers (PRIs) against bacterial wilt disease on tobacco caused by *Ralstonia solanacearum*

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The potency of plant resistance inducers (PRIs) against bacterial wilt disease on tobacco caused by *Ralstonia solanacearum*

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Abstract. The tobacco plant (*Nicotiana tobaccum* L) is one of the most valuable crops in Jember, Indonesia. One of the destructive diseases of tobacco is bacterial wilt disease caused by *Ralstonia solanacearum*. Plant resistance inducer (PRI) agents such as *Pseudomonas fluorescens*, flagella, and salicylic acid are known to have the potency to control plant pathogens by inducing a mechanism of resistance in the plant. However, there is still no study comparing their effectiveness in controlling bacterial wilt disease. This research aimed to study the effectiveness of each PRI in controlling tobacco bacterial wilt disease. The molecular assay using Polymerase Chain Reaction (PCR) confirmed that FTb4 bacteria is *R. solanacearum* and used either as inoculum or as a PRI flagella source. In addition, *P. fluorescens* IC1 was isolated from a pepper plant rhizosphere in Jember. PRIs (Isolate IC1, FTb4 flagella, and salicylic acid) were applied to control bacterial wilt disease. In vivo results showed that treatment of PRIs with medium-resistant criteria at 7 days before pathogen inoculation successfully suppressed disease incidence up to 90-93 % and disease severity up to 33.33 %. Usage of PRIs on tobacco plants increased peroxidase activity and total phenol production, indicating that PRIs induced plant resistance.

1. Introduction

The tobacco (*Nicotiana tabacum* L. belongs to the family of Solanaceae) is a valuable plantation crop produced in several regions in Indonesia. The decline in tobacco production is caused by various factors, including the internal factors such as the attack of pests and diseases that affect the tobacco production [1]. On the other hand, the bacterial wilt disease caused by *Ralstonia solanacearum*, a soilborne pathogen, is one of the major diseases in tobacco, globally causing yield loss for about 11% of tobacco plantation, represent economic loss up to USD. 9.4 million [2]. The infected tobacco plant will show several symptoms such as the leaves turn yellow prematurely, the brown discoloration of the xylem area, and wilt [3]. Induction of plant resistance is an effort to increase defense strategy in plants to prevent pathogen infection and development in plants [4]. In addition, the induction of plant

resistance can be done by applying biotic and abiotic agents, called plant resistance-inducing agents or plant resistance inducers (PRIs), including phage-infection bacteria, rhizobacteria, and microbe cell components such as flagella. However, there is limited study to compare the effectiveness of PRIs against bacterial wilt disease on tobacco. Therefore, this report shows the effectiveness of PRIs in controlling bacterial wilt disease in tobacco plants and the response of tobacco plant treated with the PRIs.

2. Material and Methods

2.1. Bacterial isolates

The *R. solanacearum* FTb4 was isolated from a wilt-diseased tobacco plant grown on cassaminoacid peptone glucose (CPG) agar plate containing 0.01% of 2,3,5-Triphenyl-tetrazolium chloride (TZC) for 48 hours at 28 °C. A single cloudy white, fluidal, viscous, irregular shape and a non-translucent colony was then transferred to a 4.5 mL of CPG broth for purification. Routinely, the isolate was grown in CPG broth [5]. Clarification of *R. solanacearum* was done through polymerase chain reaction procedure using pair primers to detect the presence of FliC and phcA sequences [6]. *Pseudomonas fluorescens* IC1 was isolated from the soil of the rhizosphere of chili. The soil extract was then streaked on King's B medium and incubated for 24 hours at 28 °C. A single fluorescent colony under ultraviolet (UV) light was picked up using a sterile toothpick and transferred into liquid King's B medium for further use [7].

2.2. Isolation of flagella

The crude flagella were collected from *R. solanacearum* FTb4 cell culture. Briefly, a single colony was spread on a CPG plate and incubated for 24 hours at 28ºC. About an eight milliliter of cold-sterile distilled water was added to re-suspend the cells, followed by passing through a 22-gauge syringe ten times before centrifugation at 4 °C for 20 minutes. The supernatant was then filtered using a 0.45 um membrane filter and stored at -20ºC for further use [8].

2.3. Plant resistance inducers (PRIs) preparation

In this study, the PRIs were flagella, *P. fluorescens*, and salicylic acid. The flagella solution (extracted from 1.9×10^8 cfu/ml) was prepared from the crude stock, as mentioned above. The suspension of *P. fluorescens* IC1 was prepared from liquid culture suspended in sterile water to a final concentration of 10^8 cfu/mL. Simultaneously, the salicylic acid solution was prepared in a stock of 100 ml of sterile distilled with a concentration of 10 mM. For treatment, a stock solution was diluted ten times in sterile water [9].

2.4. Bioassay and efficacy test against R. solanacearum on tobacco

The tobacco plants used in the study were Besuki varieties. Tobacco seeds were grown in a pot until 45 days old before treatment. About 20 mL of each PRI was poured in the soil (around the tobacco root) following by the inoculation of *R. solanacearum* FTb4. The PRIs were treated 7 days before and 7 days after inoculation with *R. solanacearum*. The pathogen was inoculated by injecting 200 microliter of *R. solanacearum* suspension just between the first and the second leaves from the bottom [10]. The positive control was tobacco inoculated with pathogen without PRIs, while negative control was a tobacco plant treated with sterile water without pathogen.

2.5. Determination of total phenol content and peroxidase activity in tobacco plants

Total phenolic content in tobacco plant was determined as mg gallic acid per gram of leaves from leaves extract as described by Addy et al [11] using Folin-Ciocalteau reagent. The hydrogen peroxidase activity in the tobacco plant was determined as a unit per mg using pyrogallol as a substrate [12].

3. Results and Discussion

3.1. Clarification of bacterial isolates

The results showed that the bacterium isolated from the diseased tobacco plant was a fluidal white colony and viscous on CPG plate (Figure 1a). This morphology feature was similar to *R. solanacearum,* as previously described by Arwiyanto [2]. In addition to the colony, the bacterium was subjected to PCR confirmation showing a specific amplicon on the agarose gel (Figure 1b). These PCR products were similar to the previous result confirming the bacterium of *R. solanacearum* that has specific flagellin C sequences (*fli*C) and *phc*A sequence, respectively [11, 13].

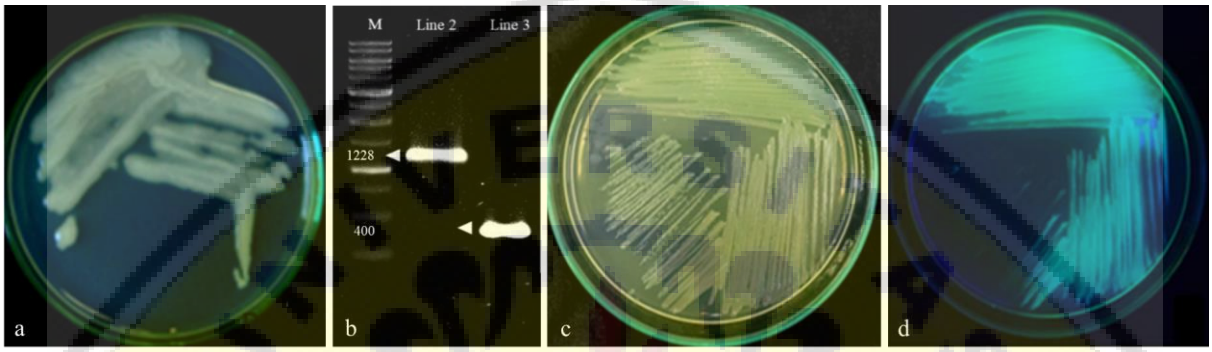

Figure 1. A bacterial colony morphology growth on CPG medium (a) and was subjected to PCR producing bands (lane 2: phcA fragment and lane 3: fliC fragment) on agarose gel (b). Morphology colony of rhizosphere bacteria on King's Medium (c) and observed under UV light (d).

3.2. Effectiveness of PRIs Application

The results showed that the application of PRIs at 7 days before inoculation reduced the disease severity on tobacco plant. The reduction was with a higher percentage of effectiveness than the application at 7 days after pathogen inoculation. However, applying PRIs for all treatments reduced the infection rate of pathogen on tobacco at 0.86 unit/day compared to the control plant at 0.28 unit/day (Table 1).

Table 1. Value and Scale of Application Effectiveness of Multiple Resistance Inducing Agents.

Control [a)]	Disease Severity (%) [b)]	Infection Rate (unit/day)	Effectiveness (%)	Effectiveness grade [c)]
Positive Control	73 a	0.8625	-	-
Negative Control	0 b	0	-	-
Salicylic Acid 10 mM (-7d)	10 b	0.2875	86	VG
Salicylic Acid (+7d)	23 b	0.2875	68	G
P. fluorescens IC1 (-7d)	10 b	0.2875	86	VG
P. fluorescens IC1 (+7d)	20 b	0.2875	73	VG
Flagella of FTb4 (-7d)	7 b	0.2875	91	VG
Flagella of FTb4 (+7d)	13 b	0.2875	82	VG

[a)] (-7d) means 7 days before inoculation, while (+7d) means 7 days after inoculation with *R. solanacearum*.
[b)] The same letter following the number indicates insignificant differences according to the Duncan test at the 5% level.
[c)] VG = Very Good (> 70%), G = Good (50-69%), P = Poor (30-49%) and NG = Not Good (<30%).

The application of *P. fluorescens* IC1 before the inoculation of the FTb4 pathogen was predicted to adapt and colonize tobacco plants' roots, consequently activating resistance gene signals. According to

Suryadi [14] treated plant root with *P. fluorescens* PF3 before planting will protect the root area and effectively inhibit the attack of *R. solanacearum* pathogens on peanuts.

Moreover, flagella have been known as an organelle of bacteria that can be used as plant elicitors to control pathogens through induced systemic resistance (ISR) mechanism [15,16]. Although the flagella cannot actively colonize the roots, it is recognized by tobacco root elicitor that triggering the resistance mechanism [17]. Besides, salicylic acid was prone to induce ISR resistance, close to FTb4 flagella and *P. fluorescens* IC1. Salicylic acid has been known as an inducer of plant resistance, influencing plant hormonal factors in protecting plants from pathogens and stress [18]. It could be absorbed by plant roots and improve resistance by influencing the biochemical processes of plants. A similar result is also shown by Bawa et al. [19] that applying salicylic acid minimizes the occurrence of *Fusarium solani* before pathogen inoculation in soybean.

3.3. Tobacco Disease Resistance Response

By applying PRIs to tobacco plants inoculated with *R. solanacearum* FTb4, the production of resistance compounds such as peroxidase and total phenol has significantly reduced as well as tobacco plant without pathogen inoculation. This result indicated that tobacco plant treated with PRIs successfully reduced the plant's biotic stress since the plant without PRIs still produced a high number of total phenol and peroxidase. In addition, the plant treated with PRIs at 7 days before pathogen had a lower number of total phenol and peroxidase at the same level as healthy plants (Figure 2).

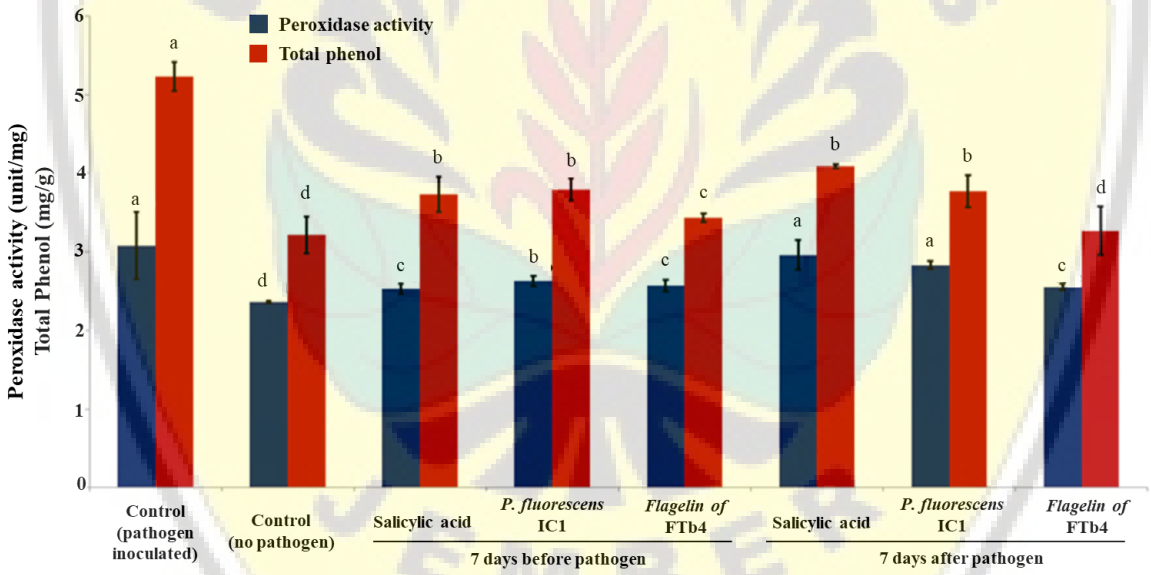

Figure 2. Peroxidase activity and total phenol in tobacco plants 20 days after pathogen inoculation.

Peroxidase activity and total phenolic were resistance compounds that play a role in increasing tobacco plant resistance. Peroxidase is an enzyme included in the PR-protein group of the PR-9 group, whose numbers increase when plants are infected, reported in the study of Van Loon et al. [20]. Peroxide is an antioxidant enzyme that functions in the ROS process as a second messenger in order to directly suppress the growth of pathogens and increase plant resistance responses by reinforcing the walls of plant cells. This condition will affect the pathogen to invade the plant cell. However, the application of induction can increase tobacco plants peroxidase content [21, 22]. In addition, the application of inducer and pathogen inoculation at the same time also has a high peroxidase activity because single inoculation of pathogens can also increase the peroxidase content [12,23]. Compared to healthy plants, plants infected with pathogens have a high degree of peroxidase. Biotic stresses such as pathogens may be the source of the increased phenol content [17]. An increase in phenol and salicylic

acid compounds in plants may serve as sources of hormones and antibiotics to suppress pathogen development [24].

4. Conclusion

In conclusion, the application of plant resistance inducers (salicylic acid, IC1 isolates, and flagella FTb4) at 7 days before pathogen inoculation effectively suppressed the growth of pathogen 90 to 93% by the medium-resistant tobacco level of resistance. These PRIs induced tobacco plant resistance against bacterial wilt disease resistance are characterized by a low total phenol content and peroxidase activity in the tobacco plant and a healthy plant.

5. References

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