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The Endophytic Bacteria, Salicylic Acid, and their Combination as Inducers of Rice Resistance Against *Xanthomonas oryzae* pv. *oryzae*

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ABSTRACT

Bacterial leaf damage or blight brought by bacteria *Xanthomonas oryzae* pv. *oryzae* (*X. oryzae* pv. *oryzae*) is considered as an extremely serious disease of rice worldwide, including Indonesia. Induced resistance using chemical and biological agents was considered as a method to control the disease. The objectives of this research were to analyze of endophytic bacteria (*Lysinibacillus sphaericus*/*L.sphaericus*) and salicylic acid as the inducers of rice resistance against *X. oryzae* pv. *oryzae*. This study used three-factorial incompletely randomized designs to study the effect of endophytic bacteria and salicylic acid on three varieties of rice. The results of the study showed that endophytic bacteria and salicylic acid induced rice resistance to *X. oryzae pv. oryzae*. This endophytic bacterium was also able to increase the plant height (AUPHGC), the number of tillers (AUNTGC), and the number of productive tillers. The application of salicylic acid at the concentrations of 10 mM was able to suppress the BLB disease and increased the number of tillers and 1000-grain weight. The result of study showed that the endophytic bacteria and salicylic acid could induce resistance of rice varities against BLB disease and increased the number of productive tillers.

INTRODUCTION

Bacterial leaf blight (BLB) caused by *X. oryzae pv. oryzae* is considered as one of the most important and very serious diseases of rice worldwide, including Indonesia (Ou, 1985). Suparyono, Sudir, & Suprihanto (2004) reported that the crop loss due to this disease was vary between 15-80 %, depending on the growth stages when the pathogen attacks and environmental factors. The development of BLB was highly affected by humidity, temperature, cultivation methods, varieties, and nitrogen fertilizer application. Among those factors, varietal resistance was the best alternative to overcome the disease. However, this technique is constrained by the pathotype development of the pathogen, which leads to resistance breakdown. Three rice varieties i.e. Conde, Angke and Impara Jete 6 were known to have a good resistance against *Xoo* (Suprihatno et al., 2011). In Indonesia, twelve *Xoo* pathotype groups has been identified with different virulence genes levels (Hifni & Kardin, 1998). Sudir, Nuryanto, & Kadi (2012) reported that pathotype III, IV, dan VIII were the dominant groups from the rice ecosystem in Java, Indonesia.

Biological control of bacterial leaf blight diseases using the application of endophytic *Bacillus* species, designated strains YC7010 has been reported (Chung et al., 2015). The bacterial leaf blight was suppressed effectively by drenching the bacterial suspension, together with the growth of rice seedlings promotion. Similar results were also reported by Lanna-filho et al. (2013) on tomato (*Xanthomonas vesicatoria*) by using endophytic *Bacillus amyloliquefaciens* and *Bacillus pumilus*. Several other endophyte species also may increase antibacterial (Strobel & Daisy, 2003), antivirus (Guo, Liang, & Zhu, 2009), antibiotic (Arunachalam & Gayathri, 2010), and antioxidant (Anuradha, Jaleel, Salem, Gomathinayagam, & Panneerselvam, 2010) activities.

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The application of chemical substances to induce plant resistance has been reported by Babu et al. (2003) who showed that induction of disease resistance in rice accelerated after treatments using acibenzolar-S-methyl (ASM). Meanwhile, Faoro & Gozzo (2015) summarized the efficacy of Chitosan (CHT) and benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) in the control of *Rhinchosporium secalis* and *Blumeria graminis f. sp. hordei*.

Furthermore, the combination of both chemical and biological elicited induced resistance in pathogen controlling has been widely studied. Resistance mechanism induction in foliar tissues on arbuscular mycorrhizal (AM) fungi colonization in soybean plants, using acibenzolar-S-methyl (ASM) as chemical elicitor, led to a significant defense reaction in the roots. At the meantime, the combination treatment composing of strain INR7 with a chemical inducer, benzothiadiazole (BTH) onto pepper elicited an induced systemic resistance response agains bacterial spot (Yi, Yang, & Ryu, 2013). To the best of our knowledge, the chemical and biological combination in pathogen controlling for bacterial leaf blight disease has never been studied. Therefore, in this study the researcher analyses the effect of combination of endophytic bacteria *Lysinibacillus sphaericus* and salicylic acid as inducers of rice resistance to *X. oryzae pv. oryzae*.

MATERIALS AND METHODS

This research was conducted from June to December 2015. The *in vivo* research was carried out in the greenhouse of Bogor Agricultural University. *In vitro* analysis was conducted in the Laboratory of Bacterial Plant Pathogens at Department of Plant Protection and Centre for Life Sciences and Biotechnology at Bogor Agricultural University, Bogor.

This study consists of three factors in a completely randomized design comprising varieties namely IR64, Ciherang, Conde, endophytic bacteria of *L. sphaericus* (treatment and non treatment) and the concentration of salicylic acid 0 and 10 mM. The susceptible variety is IR64, the moderate variety is Ciherang, and the resistant variety is Conde. Data of the study were analyzed in analysis of variance, and results of the study were analyzed by F test, while the difference between treatments was tested by Duncan's Multiple Range Test at 5 % level using SAS software version 9.2.

Plant Material and Application of Endophytic Bacteria

Three rice seeds of different varieties i.e. IR 64, Ciherang, and Conde were disinfected with sodium hypochlorite for a few minutes and repeatedly rinsed with sterile water for three times. Then the seeds were air-dried and sterilized by hot water treatment of 55 °C for 20 minutes subsequently. Afterwards, the rice seeds were then soaked in a 24-hour old bacterial suspension of 10^8 cfu ml^{-1} overnight (or for approximately 16 hours). Next, the rice seeds were planted in a black plastic tray containing a mixture of soil and manure (1:1). After 14 day-old plants, they were then moved to pots consisting of a sterile soil and manure mixture in a 1: 1 ratio. The endophytic bacteria was applied by flushing 10^8 cfu ml^{-1} of bacteria suspension at 50 ml for each plant during transplanting process. These isolates have been identified as *L. sphaericus* (Collection of the Laboratory of Bacterial Pathogens Plant, Bogor Agricultural University) (Parida, Damayanti, & Giyanto, 2014).

Application of Salicylic Acid and Inoculation of *X. oryzae* pv. *oryzae*

Salicylic acids with the concentration of 10 mM at 20 mL (HOC_9H_8COOH, Merck KGaA cas No. 66-72-7) was sprayed onto each plant every morning for 40 days. At the 43rd day after sowing they were ready for the the inoculation of *X. oryzae* pv. *oryzae* (pathotype IV), the leaf margins were clipped in two to three places with a scissor surface that was already dipped in the 10^7 cfu ml^{-1} of bacteria suspension previously. The high humidity were maintenanted by wrapping the whole plants with transparent plastic for 3-4 days.

Analyses of The Effect of Endophytic Bacteria and Salicylic Acid on BLB and The Growth of Rice Plants

Observations of BLB disease on latent period, disease severity, infection rate (Van Der Plank, 1963), and area under disease progress curve/AUDPC (Madden, Hughes, & van den Bosch, 2007) were carried out. The observations of disease severity were done by observing the severity score every 5 days after inoculated. The crops damage criteria (modified from IRRI 1996) based on percentage of leaf area infected by BLB were as follows: 0;0 < x ≤ 1 %, 1; 1 < x ≤ 5 %, 2; 5 < x ≤ 15 %, 3; 15 < x ≤ 25 %, 4; 25 < x ≤ 50 %, 5; > 50 %.

The observations of the plant growth and yield were carried out on the areas under plant height growth curve/AUPHGC, number of tillering growth curve/AUNTGC (Cooke, 1998), the number of productive tiller, and 1000-grain weight. The analyses of defense enzymes were carried out on peroxide enzyme, (Hammerschmidt, Nuckles, & Kuć, 1982), polyphenoloxidase (Mallick & Singh, 1980), β-1,3 glucanase (Pan, Ye, & Kuć, 1991), and phenylalanineammonia-lyase (Singh & Prithiviraj, 1997) before and after the inoculation of *X. oryzae* pv. *oryzae*.

RESULTS AND DISCUSSION

Effects of Application Endophytic Bacteria and Salicylic Acid on Bacterial Leaf Blight in Three Rice Varieties

The analysis of variance for the varieties, endophytic bacteria and salicylic acid, and their interactions in three rice varieties in induced resistance to *X. oryzae* pv. *oryzae* is presented in Table 1. Table 1 shows that the influence of varieties, endophytic bacteria is affected by latent period and AUDPC, while salicylic acid has no significant effect. The interaction between varieties and endophytic bacteria *L. sphaericus* significantly affects AUDPC, but the rate of infection and latent period is not significant. The interaction between varieties and salicylic acid significantly affects AUDPC, but the latent period and the rate of infection are not significant. Similarly, the interaction between the endophyte *L. sphaericus* and salicylic acid significantly influences the latent period and AUDPC, while the rate of infection is not significant. The interaction between endophytic bacteria and salicylic acid shows more significant effects on the progress of BLB disease.

Table 2 shows that the latent period of Conde variety is lower than IR64 and Ciherang. Likewise, the value of these AUDPC from this variety is smaller than the other varieties. The results shows that the rate of infection and treatment of AUDPC is affected by endophytic bacteria, but it is not influenced by salicylic acid. This is due to the varieties which have an innate resistance to inhibit BLB disease progression. The variety of Conde is also reported to have resistant genes of Xa7 to BLB disease (Tasliah, 2012).

Endophytic bacteria of *L. sphaericus* has a significant effect on the disease progress of BLB. The endophytic bacteria is able to suppress the infection rate of the disease and the AUDPC values are small compared to controls (Table 2). The Ciherang and IR64 varities which are susceptible to moderate resistant are induced from susceptible to moderate and moderate to resistant in this research. The induced resistance of both varieties is built by the bacterium *L. sphaericus* as the main regulator. According to Gnanamanickam, Vasudevan, & Velusamy (2004), some strains of *Bacillus* sp were able to stop the growth of *X. oryzae* pv. *oryzae* colonies and reduced the yields loss in the rice variety of IR24 and Jyothi of 36-59 % and 21-60 %.

Table 1. The analyses of variance of the application effects of endophytic bacteria and salicylic acid in three varieties of rice plants on bacterial leaf blight diseases caused by *X. oryzae* pv. *oryzae* on rice

Variables	A	B	C	AB	AC	BC	ABC
Latent period	0.78	0.05	0.00**	0.09	0.92	0.01**	0.48
Infection rate	0.00**	0.95	0.23	0.20	0.03*	0.93	0.48
AUDPC	0.00**	0.14	0.00**	0.00**	0.00**	0.00**	0.34

Remarks: A = endophytic bacteria of *L. sphaericus*; B = salicylic acid; C = rice variety; * and ** indicate significant at the probability of 0.05 and 0.01 levels, respectively.

Table 2. The effects of endophytic bacteria, salicylic acid, and rice variety on latent period, infection rate, and AUDPC of bacterial leaf blight diseases

Treatment	Levels	Latent period	Infection rate	AUDPC
Endophytic bacteria	Control	6.00	0.94 a	1 040.72 a
	L. sphaericus	5.44	0.53 b	861.68 b
Salicylic Acid	0 mM	5.78	0.73	978.73
	10 mM	6.17	0.74	923.67
Variety	IR64	5.75 b	0.76	996.33 a
	Ciherang	5.67 b	0.87	1 002.4 a
	Conde	6.50 a	0.59	854.85 b

Remarks: According to Duncan’s Multiple Range Test, values with different letters in a column are much different.

The interaction effects of the variety and endophyte are significant on the disease progress of BLB. The rate of infection of Ciherang variety treated with endophytic bacteria *L. sphaericus* is lower than those without endophytes. Similarly, the AUDPC value of endophytic bacteria treatment in the same variety is lower than the control.

This condition is allegedly caused by compatibility between host genotype and endophyte in triggering resistance induction in rice plants so that susceptible and moderates varieties could improve their level of resistance on BLB disease. The endophyte bacteria of *L. sphaericus* have the ability to introduce resistance in rice against the blight disease which is caused by endophyte that has already colonized the entire plant and inhibited the growth of this pathogen and the pathogenesis process in the plants tissues. The endophytes induced resistance through the elicitation of plant defenses compounds such as antibiotics and phytoalexin to inhibit dispersal of the pathogen. A recent research reported that bioactive phytochemical compounds produced by endophytes were potential to be biological control agents (Joseph & Priya, 2011). Allegedly, the endophytic bacteria of *L. sphaericus* produce bioactive compounds that could suppress the disease progression of BLB. According to Melnick, Suárez, Bailey, & Backman (2011), *L. sphaericus* endophytic bacteria was isolated from the cocoa plant as a potential biological agents in the control of cocoa pod disease (*Monilia fructicola*).

Salicylic acid does not significantly affect the disease progression of BLB (Table 2). However, the results showed that 10 mM salicylic acid treatments were more effective in hindering the progression of BLB disease compared with controls. Salicylic acid as a regulator of the defense system of plants can activate plant defense compounds needed by plants to inhibit the growth of this pathogen. Vlot, Dempsey, & Klessig (2009) reported that salicylic acid has an important role in inducing plant resistance through the activation of defense compounds needed by plants to inhibit the growth of pathogens. SA-mediated defense responses promote resistance against the rice bacterial blight pathogen.

The interaction effects of varieties and salicylic acid are significantly shown on disease progression of BLB (Table 3). By spraying 10 mM salicylic acid on IR64 variety, it could reduce the disease. This suggests that IR64 has proved to be induced resistance through the application of salicylic acid to inhibit dispersal of BLB disease. Salicylic acid is able to suppress the disease progression on this disease because of the ability of salicylic acid to trigger plant defense signal to form compounds such as the defense of the genes of PR-1 and phytoalexin to suppress the disease. The results of pre-test study of various concentrations of salicylic acid (0, 5, 10, 15, and 20 mM) revealed that 10 mM was the best concentration to induce resistance of rice against *X. oryzae* pv. *oryzae*.

Table 3. The effects of interaction among endophytic bacteria, salicylic acid, and rice varieties on latent periods, infection rate and AUDPC of bacterial leaf blight on rice

Variety	Endophytic bacteria		Salicylic acid	
	Control	**L. sphaericus**	**0 mM**	**10 mM**
		Latent period (days after inoculation)		
IR64	5.83	5.67	5.67	5.83
Ciherang	5.67	5.67	5.17	6.17
Conde	6.50	6.50	6.50	6.50
		Infection rate		
IR64	0.1008 ab	0.50 c	0.92	0.59
Ciherang	0.1272 a	0.47 c	0.82	0.92
Conde	0.0554 c	0.62 bc	0.47	0.71
		AUDPC		
IR64	1 200.17 a	792.50 c	1 155.50 a	837.17 b
Ciherang	1 237.83 a	767.00 c	1 110.50 a	894.33 b
Conde	684.17 c	1 025.53 b	670.20 c	1 039.50 b

Remarks: According to Duncan's Multiple Range Test, values with different letters in a column are much different.

Generally, salicylic acid content in rice plants is so high that if the spraying of these hormones exceeds the concentration required by the plant, it will have an antagonistic effect on the induction of plant resistance. According to Silverman et al. (1995), endogenous salicylic acid content in rice plants amounted to 30-40µg. The results showed that when the concentration of salicylic acid increased, it would affect the acceleration of BLB progress. Salicylic acid application is potential to improve plant resistance, however, the effect is highly dependent on the compatibility between the host genotype and salicylic acid. The incompatible of variety with the applications of salicylic acid can pose a risk of phytotoxicity, but, with compatible varieties it will increase the plant resistance and promote the plant growth. Hoerussalam, Purwantoro, & Khaeruni (2013), stated that C20 line increased resistant status of being susceptible to be moderate resistant to resistant. Furthermore, the resistance is still visible to the S1 generation descendant. This strains most responsive to these four kinds of elicitor (Bio1, Bio2, Abio1, and Abio2) and the increased status of susceptible to moderately resistant and become resistant to downy mildew (*Peronosclerosopora maydis*).

Table 4. The effects of interaction between endophytic bacteria and salicylic acid on latent periods, infection rate and AUDPC of bacterial leaf blight on rice

Endophytic bacteria	Salicylic acid	
	0 mM	**10 mM**
	Latent period (dai)	
Control	5.56 b	6.00 ab
L. sphaericus	6.44 a	5.89 ab
	Infection rate	
Control	0.93	0.53
L. sphaericus	0.95	0.53
	AUDPC	
Control	1 154.44 a	803.02 c
L. sphaericus	927.00 b	920.33 b

Remarks: According to Duncan's Multiple Range Test, values with different letters in a column are much different.

The interaction effects between the endophytic bacteria and salicylic acid is significantly shown on disease progression of BLB (Table 4). Both of these inducers potentially affect the induction of plant resistance singly or together as compared with controls. The results showed that the treatment with endophytic bacteria individually was able to suppress the BLB. The latent period of the treatment of Ciherang and 10 mM salicylic acids was not significantly different from controls. The treatment with endophytic bacteria and salicylic acid singly on latent period was longer compared to the control. The rate of infection did not significantly show disease progression of BLB, but the lowest infection rate was obtained in the treatment of endophytic bacteria and salicylic acid compared to the controls.

According to Tamaoki et al. (2013), the application without jasmonic acid, salicylic acid activated the defense system against blast disease of rice plants, otherwise if jasmonic acid was activated then the effect could suppress the salicylic acid.

The research indicated that the synergistic effects occured between endophytic bacteria of *L. sphaericus* and salicylic acid induced the plant resistance against BLB. However, the mechanism between endophytic bacteria of *L. sphaericus* and salicylic acid together suppressesing the disease progression of BLB was unknown. Mur, Kenton, Atzorn, Miersch, & Wasternack (2006) said that the gene expression of jasmonic acid (PDF1.2 and Thi1.2) and salicylic acid (PR-1 on tobacco) would increase if they were applied in low concentrations and would have a synergistic effect. Allegedly, many possibilities may occur if the application of salicylic acid and endophytic bacteria given in low concentrations causes synergism in inducing plant resistance to BLB disease.

Growth of Response and Yield of Three Rice Varieties that was Induced Resistance with Endophytic Bacteria and Salicylic Acid

The analysis of variance for the effects of varieties, salicylic acid, endophytic bacteria and their interactions can be seen in Table 5, while the significance tests of the varieties, salicylic acid and endophytic bacteria and their interactions on growth and crop of rice varieties are presented in Tables 6, 7, and 8.

The effect of varieties on the growth of rice plants affects plant height (AUPHGC) and the number of productive tillers (Table 6). The plant height of Ciherang and Conde varieties increased compared with IR64. The conde variety had higher total productive tillers. The results showed that IR64 and Ciherang varieties had a better growth response and yield through the production of 1000 grain weight. It is because the two varieties allocating more energy are directed to the production, while Conde tends toward the plant resistance. The resistant varieties tend to allocate costs for plant defense physiologically, while susceptible varieties are directed to the production of yields Walters & Heil (2007).

Table 5. The analysis of variance of the application effects of endophytic bacteria and salicylic acid in three varieties of rice on the rice growth and yield

Variables	A	B	C	AB	AC	BC	ABC
Plant height (AUPHGC)	0.00**	0.49	0.00*	0.00**	0.03*	0.04*	0.17
AUNTGC (number of tiller)	0.92	0.00**	0.00**	0.00**	0.00**	0.23	0.19
Number of productive tillers	0.00**	0.75	0.00**	0.08	0.50	0.00**	0.71
1000-grain weight	0.99	0.00**	0.10	0.74	0.30	0.92	0.99

Remarks: A = Endophytic bacteria *L. sphaericus*; B = salicylic acid; C = rice variety; * and ** indicate significant at the probability of 0.05 and 0.01 levels, respectively.

Table 6. The effects of endhophytic bacteria, salicylic acid, and rice variety on AUPHGC, AUNTGC, the number of productive tillers and 1000-grains weight

Treatment	Levels	AUPHGC* (unit/week)	AUNTGC*	Number of productive tillers*	1000-grain weight (g)*
Endophytic bacteria	Control	696.08 a	189.31 a	16.17 b	23.75
	L. sphaericus	660.77 b	199.41 b	17.61 a	23.28
Salicylic acid	0 mM	681.02	187.49 b	16.83	23.91 a
	10 mM	676.79	201.23 a	16.94	23.11 b
Varieties	*IR64*	661.69 c	195.33	17.67 a	23.96
	Ciherang	676.02 b	193.53	15.00 b	23.67
	Conde	707.61 a	194.53	18.00 a	22.92

Remarks: According to Duncan's Multiple Range Test, values with different letters in a column are much different. (P ≤ 0.05); * = corrected data with control (without *X. oryzae* pv. *oryzae*).

Table 7. The effects of interaction between endophytic bacteria, salicylic acid, and rice varieties on AUPHGC, AUNTGC, the number of productive tillers and 1000-grains weight

Variety	Endophtic bacteria		Salicylic acid	
	Control	*L. sphaericus*	0 mM	10 mM
		*AUPHGC (unit)**		
IR64	664.53 c	638.85 cd	669.51 c	633.87 d
Ciherang	631.01 d	739.03 a	671.64 c	680.39 bc
Conde	704.78 b	710.23 b	701.91 ab	713.10 a
		*AUNTGC**		
IR64	196.83 bc	196.83 bc	184.80 b	205.87 a
Ciherang	172.01 d	216.05 a	170.37 c	216.70 a
Conde	202.08 b	186.35 c	207.30 a	181.13 bc
		Number of productive tillers		
IR64	17.00	18.33	17.17	18.50
Ciherang	14.50	15.50	15.83	15.33
Conde	17.00	19.00	17.67	17.17
		1000-grain weight (g)		
IR64	23.50	23.53	23.67	23.75
Ciherang	23.33	24.00	23.67	24.17
Conde	23.75	22.83	23.25	22.58

Remarks: According to Duncan's Multiple Range Test, values with different letters in a column are much different. (P ≤ 0.05), * = corrected data with control (without *X. oryzae* pv. *oryzae*).

Table 8. The effects of interaction between endophytic bacteria and salicylic acid on AUPHGC and the number of productive tillers

Endophytic bacteria	Salicylic Acid	
	0 mM	10 mM
*AUPHGC**		
Control	655.08 c	706.96 a
L.sphaericus	666.47 bc	686.11 b
*Number of productive tillers**		
Control	16.67 bc	17.00 b
L. sphaericus	15.67 c	18.22 a

Remarks: According to Duncan's Multiple Range Test, values with different letters in a column are much different. (P ≤ 0.05), * = corrected data with control (without *X. oryzae* pv. *oryzae*).

The interaction effects between the varieties and endophyte could stimulate the growth of plant height and number of tillers of the Ciherang variety compared with both other varieties. Treatments using endophyte bacteria of *L. sphaericus* increased the number of productive tillers more than without endophyte (Table 6). This suggested that the endophytic bacteria of *L. sphaericus* also acts as a promoting growth bacteria of rice plants.

Some species of endophytic bacteria are able to produce hormones that stimulate plant growth bacteria. Endophytic bacteria of *Streptomyces* sp. EN1 straining isolated from a medicinal plant are able to produce phytohormones IAA (Lin & Xu, 2013). Allegedly, the endophyte bacteria of *L. sphaericus* has a role as plant growth promoting bacteria of rice plants on Ciherang variety so that the 1000-grain weight of this variety is better than the other varieties. Although Conde variety has a larger number of productive tillers, the production declines more than the other two varieties. This is conceivably because of the variety of Conde prefers to use its energy resources for defense, while the two other varieties use their energy directly for photosynthesis and carbohydrate production.

Spraying of 10 mM salicylic acid is able to increase the number of tillers (AUNTGC) and the weight of 1000 grain (Table 6). Salicylic acid is known to increase the photosynthesis and production of carbohydrates required by plants. According to Hayat, Q., Hayat, S., Irfan, & Ahmad (2010), the attributes of salicylic acid can stimulate plant physiology and biochemistry processes such as nutrient uptake, cell elongation, cell division, regulation of source/sink, enzymatic activity, protein synthesis, photosynthesis activity and increase the antioxidant capacity of the plant.

The interaction effects between the varieties and salicylic acid is able to increase the plant height and number of tillers (Table 7). This is caused by the influence of host genotype and salicylic acid which affect the plant growth and increase yields by increasing the number of productive tillers and 1000-grain weight. According to Nagasubramaniam, Pathmanabhan, & Mallika (2007) the application of 100 mM salicylic acid can increase not just the plant height, but the leaf area, plant growth rate, and total dry matter production on baby corn.

The interaction effects between the endophytic bacteria of *L. sphaericus* and salicylic acid are able to increase the growth and yield (Table 8). The number of productive tillers of this treatment has increased compared to the controls. This was caused by the symbiosis between the two inducer to stimulate growth of the rice plant. Mattos et al. (2008) said that *Burkholderia kururiensis* endophytic bacteria found in rice plants act as a plant growth promoting bacteria and increase the production of dry beans. Endophytic fungal symbiosis and exogenous SA application assist plants in relieving the negative effects associated with osmotic stress through the decrease of biomass losses when compared to non-inoculated plants. These findings suggest that the application of SA application has a positive effect on microbial colonization whilst its combination causes plant growth to be reprogramed under various periods of drought stress (Khan et al., 2013).

The Activity of Enzymes in Plant Defense in three Rice Varieties that were Induced Resistance with Endhopytic Bacteria and Salicylic Acid

The activities of each enzyme in the plant defense inducing three rice varieties resistance to BLB disease are presented in Table 9. The results showed that the application of salicylic acid and endophytic bacteria indicated an increase of enzymes activities after pathogen exposure. The activities of peroxidase, β-1,3-glucanase and PAL increased after the inoculation on the combined treatment of Ciherang and IR64 varieties and 10 mM salicylic acid and endophytic bacteria of *L. sphaericus* (Table 9).

Table 9. The effects of endophytic bacteria, salicylic acid in three rice varieties on the activity of enzyme peroxidase, polyphenoloxidase, β,1.3-glucanase, and Phenylalanine-ammonia lyase before and after inoculation of *X. oryzae* pv. *oryzae*

Treatment	Plant defense enzymes activities (unit activities enzymes/UAE)							
	Before inoculations				After incoulations			
	Peroxidase	Polyphenoloxidase	β-1.3-glucanase	PAL	Peroxidase	Polyphenoloxidase	β-1.3-glucanase	PAL
A1B0C0	0.0029	0.001	0.007	0.047	0.0020	0.000	0.003	0.032
A1B1C0	0.0016	0.053	0.023	0.040	0.0022	0.015	0.013	0.025
A1B0C1	0.0021	0.098	0.004	0.024	0.0016	0.048	0.010	0.027
A1B1C1	0.0028	0.028	0.007	0.036	0.0086	0.100	0.007	0.039
A2B0C0	0.0054	0.018	0.014	0.079	0.0014	0.006	0.006	0.031
A2B1C0	0.0071	0.016	0.011	0.029	0.0042	0.028	0.008	0.065
A2B0C1	0.0031	0.041	0.010	0.053	0.0043	0.024	0.011	0.021
A2B1C1	0.0032	0.054	0.013	0.036	0.0049	0.055	0.011	0.045
A3B0C0	0.0029	0.000	0.009	0.036	0.0026	0.003	0.007	0.027
A3B1C0	0.0022	0.015	0.002	0.029	0.0012	0.008	0.009	0.047
A3B0C1	0.0049	0.006	0.008	0.055	0.0019	0.000	0.008	0.030
A3B1C1	0.0029	0.033	0.004	0.029	0.0013	0.006	0.018	0.094

Remarks: A = rice variety: A1=IR64, A2=Ciherang, A3=Conde; B = endophytic bacteria: B0= non treatment, B1= treatment; C = salicylic acid: C0=0 mM, C1=10 mM

This was caused by both inducers that could enhance the accumulation of PR-protein compounds and other defense compounds in both varieties. The indicator of a systemic induction of resistance among them accumulates formation pathogenesis of related protein (PR-proteins) (Chen, Belanger, Benhamou, & Paulitz, 2000). The PR-protein group commonly known among other is peroxidase (Ramamoorthy, 2001). The peroxidase function is to enhance the cell wall degradation enzymes produced by pathogens through the formation of structural proteins on the cell wall. Peroxidase is an enzyme that serves as a catalyst in the final stage of the process of biosynthesis of lignin and hydrogen peroxidase. Several other types of enzymes have reported to increase their activities after receiving treatment with biological agents, such as peroxidase, phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (Chen, Belanger, Benhamou, & Paulitz, 2000). In this study, the induction of resistance that occurs was affected by the enzymatic activity causing susceptible and moderate varieties and capable of induction resistance to BLB disease. Furthermore, the inducer could also spur the growth and yields of rice plants.

CONCLUSION

The treatment of endophytic bacteria of *L. sphaericus* was proven to be effective in inducing plant resistance to BLB disease. There was an interaction between the varieties and endophytic bacteria of *L. sphaericus* in inducing the plant resistance to BLB disease. Similarly, the interaction between the varieties and salicylic acid could induce resistance of rice plants against pathogens. The endophytic bacteria and salicylic acid were found to be effective as inducers of the rice plant resistance to BLB disease as well as plant growth promoting.

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