J. Microbiol. Biotechnol. (2016), 26(5), 938–945
http://dx.doi.org/10.4014/jmb.1510.10016

jmb

Screening Rice Cultivars for Resistance to Bacterial Leaf Blight

Agaba Kayihura Fred[1], Gilang Kiswara[2], Gihwan Yi[3]*, and Kyung-Min Kim[2]*

[1]*Department of Food Security and Agricultural Development, College of Agriculture and Life Science, Kyungpook National University, Daegu, 41566, Republic of Korea*
[2]*Division of Plant Biosciences, School of Applied Biosciences, College of Agriculture and Life Science, Kyungpook National University, Daegu, 41566, Republic of Korea*
[3]*Department of Farm Management, College of Agriculture and Life Science, Kyungpook National University, Gyeongbuk 39061, Republic of Korea*

Received: October 8, 2015
Revised: February 2, 2016
Accepted: February 11, 2016

First published online
February 12, 2016

**Corresponding authors*
K.-M.K.
Phone: +82-539505711;
Fax: +82-539586880;
E-mail: kkm@knu.ac.kr
G.Y.
Phone: +82-539508338;
Fax: +82-543836715;
E-mail: gihwan@knu.ac.kr

pISSN 1017-7825, eISSN 1738-8872

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious threats to rice production. In this study, screening of rice for resistance to BLB was carried out at two different times and locations; that is, in a greenhouse during winter and in an open field during summer. The pathogenicity of *Xoo* race K1 was tested on 32 Korean rice cultivars. Inoculation was conducted at the maximum tillering stage, and the lesion length was measured after 14 days of inoculation. Five cultivars, Hanareum, Namcheon, Samgdeok, Samgang, and Yangjo, were found to be resistant in both the greenhouse and open-field screenings. Expression of the plant defense-related genes *JAmyb*, *OsNPR1*, *OsPR1a*, *OsWRKY45*, and *OsPR10b* was observed in resistant and susceptible cultivars by qRT-PCR. Among the five genes tested, only *OsPR10b* showed coherent expression with the phenotypes. Screening of resistance to *Xoo* in rice was more accurate when conducted in open fields in the summer cultivation period than in greenhouses in winter. The expression of plant defense-related genes after bacterial inoculation could give another perspective in elucidating defense mechanisms by using both resistant and susceptible individuals.

Keywords: Bacterial leaf blight, rice, resistance, susceptibility, pathogenicity

Introduction

Owing to an increasing world population, expected to reach eight billion people by 2020 [14], the world's most challenging problem is to feed the growing population; as the population increases, food production decreases owing to a lack of suitable land for crop cultivation. However, rice breeders face different environmental challenges, including bacterial pathogens that cause diseases. The *Xanthomonas* genus causes serious bacterial leaf blight in many crops such as cassava and rice via a gram-negative bacterium [3, 27]. In rice, it causes annual yield losses conservatively estimated at 50% [24]. This bacterium causes reduction in total dry matter weight of rice, poor maturation, and broken grain during milling. Bacterial leaf blight (BLB) is a vascular disease that causes a white-yellow or tannish-grey discoloration in the rice crop along the veins, leaf margins, and leaf blades, and these lesions may extend to the sheath [10]. There is some research directed at understanding the principles underlying the interaction between the pathogen and its host, leading to either a compatible or an incompatible disease reaction. When rice is infected by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), although the symptoms of the disease may be observed at the tillering stage, the disease may continue to increase as the plant grows. It was observed that rice plants at less than 21 days old are more susceptible to the disease and that the bacteria may favor temperatures at 28–34°C for growth. The improvement of host resistance and the application of chemical and biological measures have been used for the control of BLB [2]. However, owing to the breakdown of resistance in many resistant cultivars, their useful lifespan is only a few years. Therefore, it is important to screen rice for bacterial leaf blight resistance in order to assess the diversity within the germplasm and

to provide information about resistance/susceptibility for further use in breeding practice [5]. Moreover, screening for varietal resistance based on artificial inoculation may always be conclusive because of to the presence of adequate inoculation initiating the disease [20]. In the present study, 32 Korean rice cultivars were observed for their reaction to *Xoo* race K1. Resistance to BLB in a greenhouse during winter and in a rice field during the summer rice cultivation period was screened and compared. Moreover, some genes related to disease response in plants are known; *JAmyb* is one of the genes associated with reactions to biotic and abiotic stress. The expression of *JAmyb* suggests its involvement in JA-mediated disease responses [15]. The *OsNH1* gene (also known as *OsNPR1*) is thought to be involved in down-regulation of gene expression, those also involved in photosynthetic activity. The overexpression of *OsNPR1/NH1*, a rice ortholog of *NPR1*, reinforces resistance to bacterial blight disease [6, 26]. The *OsPR10b* gene is known as an inducer of biotic and abiotic stress responses, including to pathogen infection, and transcripts of *PRP10b* also were enhanced by *Magnaporthe grisea* [19]. *OsPR1a* is highly responsive to plant wounds by cutting and exogenous application of phytohormones [1]. *OsWRKY45* has a role in regulating a defense-related response gene and it gives an enhanced blast resistance in rice [23]. Hence, the expression of these five defense-related genes in rice during *Xoo* infection was investigated.

Materials and Methods

Plant Materials and Field Trials

Thirty-two rice cultivars were used in this study. Field trials were conducted during the winter of 2014 at an experimental greenhouse at Kyungpook National University, Daegu, and during the summer of 2015 in a field at the Kyungpook National University Experimental Station, Gunwi, Korea. The seeds were force sprouted in an incubator at 28°C for 3 days and sown in a 50-hole tray. The seed trays were maintained in dark conditions in the greenhouse used for germination for 4 days and seeds were germinated. Fifteen seedlings per cultivar were transplanted in a planting row of paddy field with a planting density of 30 × 15 cm. The amount of fertilizer applied was N-P_2O_5-K_2O = 9.0-4.5-5.7 kg/10 a.

Bacterial Inoculation and Phenotype Analysis

The bacterial pathogen *Xoo* race K1 was cultured on peptone sucrose agar medium containing 2% sucrose (w/v), 2.5% peptone (w/v), 0.05% K_2PO_4 (w/v), and 0.025% $MgSO_4 \cdot 7H_2O$ (w/v) at pH 7.0. The bacterial culture was suspended in sterile water and adjusted to a concentration of OD_{600} = 1.0. Five replications of each cultivar were inoculated at the maximum tillering stage by cutting rice leaves using scissors dipped in the suspended solution [12]. The severity of BLB infection was determined by measuring the length of lesions (LL) on infected leaves at 14 days post-inoculation. Plant reactions to BLB were classified as resistant, moderately susceptible, or susceptible depending on LL, and the principle for classification of resistance in this study was determined by the range in disease severity.

Gene Expression Analyses

RNA was isolated from leaf tissue at 4 days post-inoculation using an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. A hundred milligram plant sample was flash-frozen in liquid nitrogen, ground by mortar and pestle, and put into a 2 ml microcentrifuge tube. Next, 450 µl of Buffer RLT was added, and the mixture was vortexed and incubated for 1–3 min at 56°C. The lysate was transferred to a QIAshredder homogenizer in a 2 ml collection tube, and centrifuged for 2 min at 13,000 rpm. Following centrifugation, the supernatant was transferred to a new 1.5 ml microcentrifuge tube along with 0.5 ml of 96% ethanol. The mixture was transferred to an RNeasy spin column placed in a 2 ml collection tube, centrifuged for 1 min at 13,000 rpm, and the flow-through was discarded. Similarly, 700 µl of Buffer RW1 was added to an RNeasy spin column, centrifuged for 1 min at 13,000 rpm, and the flow-through was discarded. The RNeasy spin column was washed twice with 500 µl of Buffer RPE followed by centrifugation for 1 min at 13,000 rpm and the flow-through was discarded. Finally, the RNeasy spin column was placed in a 1.5 ml collection tube and the total RNA was suspended in 30 µl of RNase-free water by centrifugation for 1 min at 13,000 rpm. Complementary DNA (cDNA) synthesis was conducted using a cDNA synthesis kit (Philekorea Technology Inc., Korea) by following the manufacturer's protocol. In a total reaction volume of 20 µl, 2–2.5 µg of total RNA was mixed with 1× cDNA synthesis mix (3 mM $MgCl_2$, 1 mM dNTPs, enhancers, and stabilizers) and 1× MMLV Reverse Transcriptase (RTase). The reaction was carried out at 42°C for 30 min. To stop the reaction, the reaction mixture was incubated at 70°C for 10 min to denature the RTase. Finally, cDNA was purified by ethanol precipitation before being suspended in nuclease-free water. Expression profiles of several plant defense-related genes (*i.e.*, *JAmyb*, *OsNH1*, *OsPR1a*, *OsPR10b*, and *OsWRKY45*) were generated using the Eco Real-Time PCR System (Illumina, USA). Relative expression levels were determined by comparing the expression level of each gene tested from the treated and control plants and normalized to Actin1 [18]. Primers (Table 1) were designed using Primer3 (http://bioinfo.ut.ee/) set at the following parameters: sequence length 18–25 bp, product size 100–200 bp, GC content 45–55%, annealing temperature 59.5–60.5°C, and bridge exon-exon junctions if the gene contained more than one exon. In total, the reaction mixture was 20 µl and contained 90 ng of cDNA template, 0.4 µM of each forward and reverse primer, and 1× qPCRBIO SyGreen Mix Lo-ROX (PCR Biosystems Ltd., London, UK). All analyses were conducted in triplicates.

Table 1. Primer sequences of the genes used for quantitative real-time PCR analysis on resistant and susceptible rice cultivars after 14 days of inoculation with *Xoo* race K1.

Gene	Primer sequence (5′-3′)	Product size (bp)
Actin1	Forward: ATCCTTGTATGCTAGCGGTCGA	118
	Reverse: ATCCAACCGGAGGATAGCATG	
OsNPR1	Forward: TGGCAGGTGAGAGTCTACGA	160
	Reverse: TCAGGAGGTGGATTTGCACC	
OsWRKY45	Forward: TCGTCAAGAACCTCGACGAC	117
	Reverse: TGCACAGCTGGTCGTACTTG	
JAmyb	Forward: TGAAGAGGACTGGGAAGAGCT	160
	Reverse: CTCCCAGGCAAATGTTGTGC	
OsPR1a	Forward: CGGAGAAGCAGTGGTACGAC	200
	Reverse: TCAGTAGGGAGATTGGCCGA	
OsPR10b	Forward: GCACCATCCACATCATGAAGC	133
	Reverse: TTGCCCACCCTGCTCTTAAC	

Results

Inoculations of rice cultivars with *Xoo* were conducted during two different seasons (*i.e.*, winter and summer 2014) and at two different locations (*i.e.*, greenhouse and open field) with the purpose of determining whether *Xoo* inoculation in different environments produced the same results. The severity of BLB, indicated by LL on leaves, was significantly lower in the greenhouse than in the open field (Table 2). The range of LL in the greenhouse screening was 0.24–3.33 cm whereas that in the open field screening was 0.32–8.42 (Fig. 1). Therefore, by visual observation of the LL, the reaction to BLB in the greenhouse only classified rice cultivars into two categories, resistant or susceptible. Five cultivars (Hanareum, Namcheon, Samdeok, Samgang, and Yangjo) with LL of less than 1 cm were classified as resistant and the remaining 27 cultivars with LL greater than 1 cm were classified as susceptible.

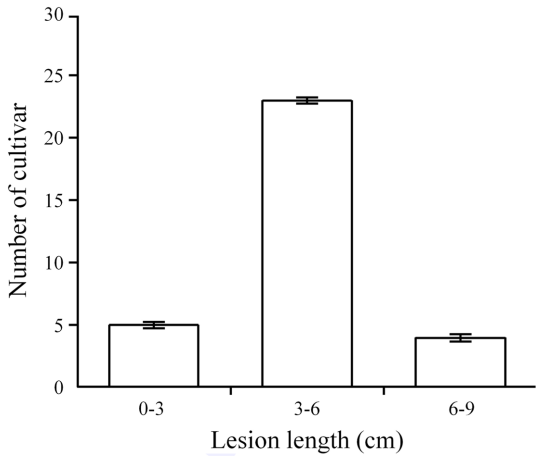

Fig. 1. Distribution of lesion length in 32 Korean rice cultivars after 14 d of inoculation with *Xoo* K1 race in open field cultivation.

Meanwhile, in the open field screening, disease severity was more obvious. Therefore, the reaction to BLB was classified into resistant (0–3 cm), moderately susceptible (3–6 cm), and susceptible (6–9 cm). The principle for classification of resistance in this study was determined by the range in disease severity. The same five cultivars were found to be resistant in both the greenhouse and open field, whereas 23 cultivars were moderately susceptible, and four cultivars were susceptible. It is also noteworthy that cultivar Joan, the most susceptible cultivar in the greenhouse, was only moderately susceptible in the open field, suggesting that greenhouse screening was not as accurate as screening in the open field.

Fig. 2 depicts the phenotypes of the five most resistant (*i.e.*, Hanareum, Namcheon, Samdeok, Samgang, and Yangjo) and susceptible cultivars (*i.e.*, Dongjin1, Hegjinju, Jeogtomi, Jinbu, and Namil) from the field inoculation. Infected leaves had yellow water-soaked lesions at the margins of the leaf blade. The lesions were parallel along the leaf and when joined together could cover the entire leaf surface. Bacterial discharge appeared from young lesions early in the morning and appeared as a milky dewdrop. As the disease progressed, the leaves dried up, showing white lesions, and the margins of the leaf blade became wavy.

Expression levels of several common genes related to

Table 2. Lesion length of 32 Korean rice cultivars after 14 days of inoculation with *Xoo* race K1 in greenhouse and open field screening.

No.	Cultivar	Greenhouse		Open field	
		Lesion length (cm)	Reaction to BLB[a]	Lesion length (cm)	Reaction to BLB[a]
1	Chokwang	1.63 ± 0.55	S	3.56 ± 0.94	MS
2	Dongjin1	2.35 ± 0.64	S	6.90 ± 0.70	S
3	Dunae	2.21 ± 0.53	S	4.06 ± 1.31	MS
4	Geumo3	1.80 ± 0.40	S	3.68 ± 1.15	MS
5	Goun	1.95 ± 0.35	S	4.34 ± 1.41	MS
6	Guru	1.70 ± 0.25	S	3.58 ± 0.94	MS
7	Haepyeong	2.45 ± 0.65	S	4.22 ± 0.95	MS
8	Hanareum	0.60 ± 0.41	R	0.32 ± 0.18	R
9	Hegjinju	1.99 ± 0.34	S	8.42 ± 1.99	S
10	Hopum	1.52 ± 0.55	S	5.32 ± 0.99	MS
11	Hwayeong	1.93 ± 0.37	S	5.36 ± 1.07	MS
12	Jeogtomi	1.87 ± 0.22	S	8.34 ± 2.80	S
13	Jinbu	1.60 ± 0.22	S	5.92 ± 1.27	MS
14	Jinmi	1.73 ± 0.16	S	3.88 ± 0.22	MS
15	Joan	3.33 ± 0.58	S	5.22 ± 1.25	MS
16	Jopyeong	1.71 ± 0.51	S	3.98 ± 1.18	MS
17	Joun	1.97 ± 0.19	S	4.54 ± 1.17	MS
18	Junam	1.79 ± 0.38	S	4.56 ± 2.09	MS
19	Namcheon	0.24 ± 0.03	R	0.68 ± 0.41	R
20	Namil	3.01 ± 0.36	S	7.38 ± 0.80	S
21	Nampyeong	1.81 ± 0.44	S	4.96 ± 1.49	MS
22	Obong	2.05 ± 0.25	S	5.26 ± 0.68	MS
23	Odea	1.60 ± 0.25	S	4.90 ± 1.10	MS
24	Samdeok	0.28 ± 0.08	R	0.40 ± 0.22	R
25	Samgang	0.53 ± 0.28	R	0.70 ± 0.21	R
26	Sangju	1.63 ± 0.20	S	3.46 ± 1.24	MS
27	Seolgaeng	2.03 ± 0.31	S	5.36 ± 1.05	MS
28	Taebong	1.87 ± 0.61	S	5.08 ± 1.26	MS
29	Undoo	1.82 ± 0.40	S	3.68 ± 0.45	MS
30	Unkwang	1.47 ± 0.14	S	3.40 ± 1.74	MS
31	Wangchal	1.96 ± 0.16	S	3.90 ± 0.65	MS
32	Yangjo	0.32 ± 0.16	R	0.36 ± 0.23	R

[a]R: resistant; MS: moderately susceptible; S: susceptible.

plant defense (*i.e.*, *OsNPR1*, *OsWRKY45*, *JAmyb*, *OsPR1a*, and *OsPR10b*) during BLB inoculation were assessed. Owing to the low severity of BLB in greenhouse-grown plants, rice cultivars from the open field were used for gene expression analysis. Five resistant cultivars (*i.e.*, Hanareum, Namcheon, Samdeok, Samgang, and Yangjo) were subjected to qRT-PCR analysis to determine which plant defense-related gene(s) are regulated during BLB infection by comparing the expression levels of the same genes in control and treated plants.

The expression of *JAmyb* in resistant cultivars was not correlated with the phenotype (Fig. 3). Its expression was higher in the cultivars Samdeok, Samgang, and Yangjo compared with control plants, but was lower in the cultivars Hanareum and Namcheon. The expression levels of *OsNPR1* were unexpectedly low in all resistant cultivars

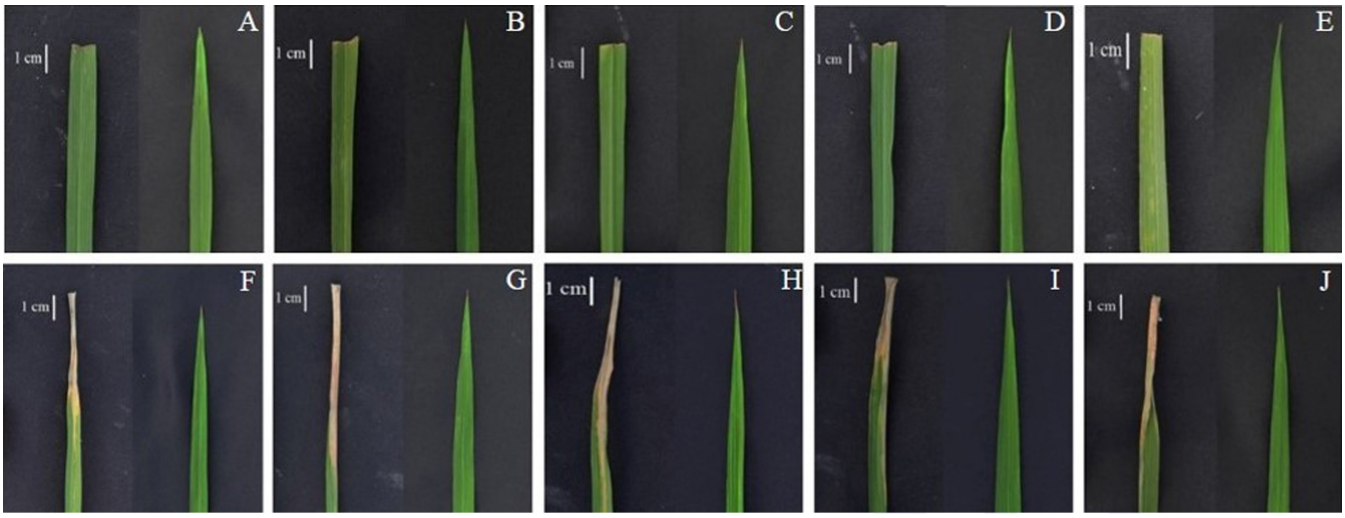

Fig. 2. Phenotypes of the five most resistant cultivars (*i.e.*, A. Hanareum; B. Namcheon; C. Samdeok; D. Samgang; E. Yangjo) and five most susceptible cultivars (*i.e.*, F. Dongjin1; G. Hegjinju; H. Jeogtomi; I. Jinbu; J. Namil), after 14 days of inoculation with *Xoo* K1 race in open field cultivation.
Leaves on the left and right in each picture are inoculated and control plants, respectively.

(Fig. 4), suggesting that this gene is not essential for BLB resistance. However, this does not mean that *OsNPR1* down-regulates a reaction to the pathogen because the relative expression in *OsNPR1* appeared randomly. The expression levels of *OsPR1a* in the resistant cultivar group were similar patterns to those noted in *JAmyb*. This gene was highly expressed in the cultivar Yangjo, whereas its expression levels in other cultivars were either lower or slightly higher than in control plants (Fig. 5). The expression levels of *OsWRKY45* in resistant cultivars were not correlated with the phenotype. Except for one cultivar (*i.e.*, Namcheon), *OsWRKY45* expression levels were lower than those in the control plants (Fig. 6). The expression levels of *OsPR10b* in all resistant cultivars were higher than those in control plants (Fig. 7). The gene was expressed twice as much, except in Yangjo, where its expression level was more than 9 times higher than in the control plants. This expression pattern indicates a positive correlation with the

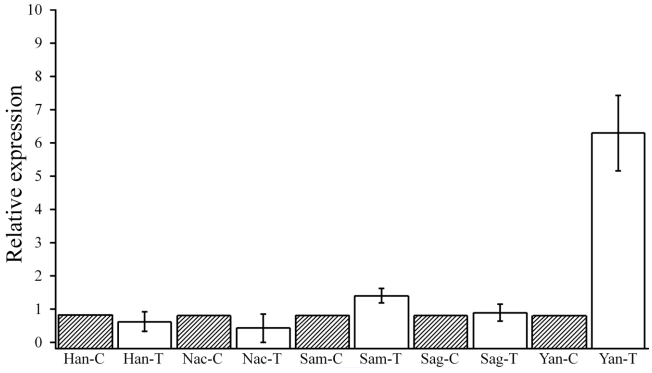

Fig. 3. Relative expression of *JAmyb* in five resistant Korean rice cultivars after 4 days of inoculation with *Xoo* race K1.
Striped bars represent control plants and bars in white represent treated plants. Han-C: Hanareum control; Han-T: Hanareum treated; Nam-C: Namcheon control; Nam-T: Namcheon treated; Sam-C: Samdeok control; Sam-T: Samdeok treated, Sag-C: Samgang control; Sag-T: Samgang treated; Yan-C: Yangjo control; Yan-T: Yangjo treated.

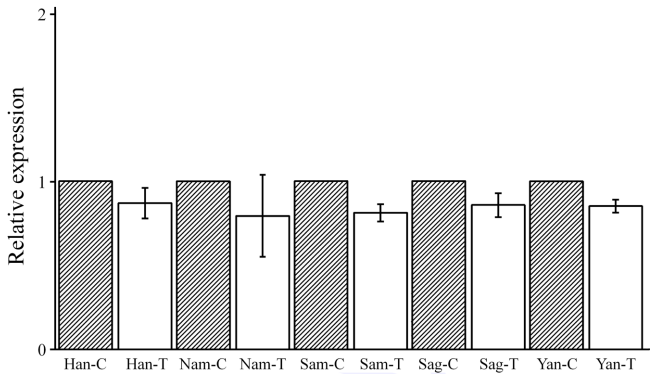

Fig. 4. Relative expression of *OsNPR1* in five resistant Korean rice cultivars after 4 days of inoculation with *Xoo* race K1.
Striped bars represent control plants and bars in white represent treated plants. Han-C: Hanareum control; Han-T: Hanareum treated; Nam-C: Namcheon control; Nam-T: Namcheon treated; Sam-C: Samdeok control; Sam-T: Samdeok treated, Sag-C: Samgang control; Sag-T: Samgang treated; Yan-C: Yangjo control; Yan-T: Yangjo treated.

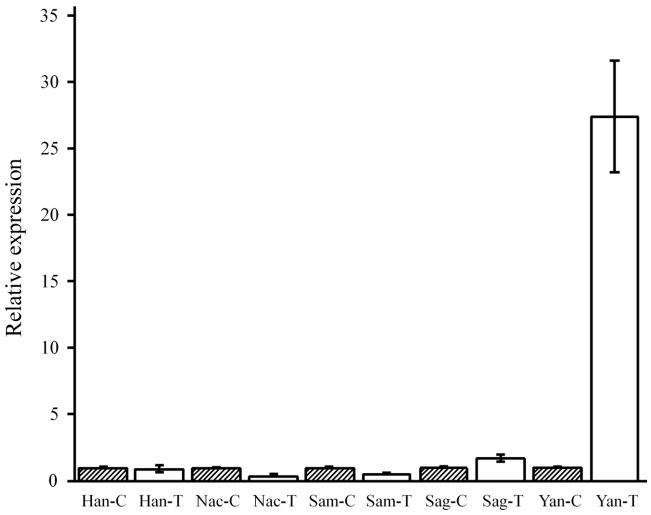

Fig. 5. Relative expression of *OsPR1a* in five resistant Korean rice cultivars after 4 days of inoculation with *Xoo* race K1.

Striped bars represent control plants and bars in white represent treated plants. Han-C: Hanareum control; Han-T: Hanareum treated; Nam-C: Namcheon control; Nam-T: Namcheon treated; Sam-C: Samdeok control; Sam-T: Samdeok treated, Sag-C: Samgang control; Sag-T: Samgang treated; Yan-C: Yangjo control; Yan-T: Yangjo treated.

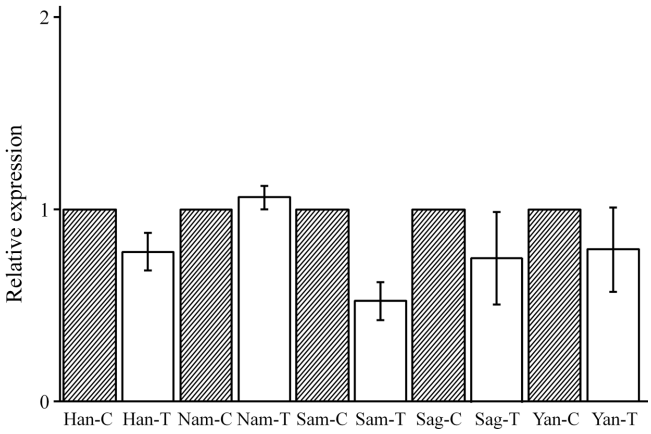

Fig. 6. Relative expression of *OsWRKY45* in five resistant Korean rice cultivars after 4 days of inoculation with *Xoo* race K1.

Striped bars represent control plants and bars in white represent treated plants. Han-C: Hanareum control; Han-T: Hanareum treated; Nam-C: Namcheon control; Nam-T: Namcheon treated; Sam-C: Samdeok control; Sam-T: Samdeok treated, Sag-C: Samgang control; Sag-T: Samgang treated; Yan-C: Yangjo control; Yan-T: Yangjo treated.

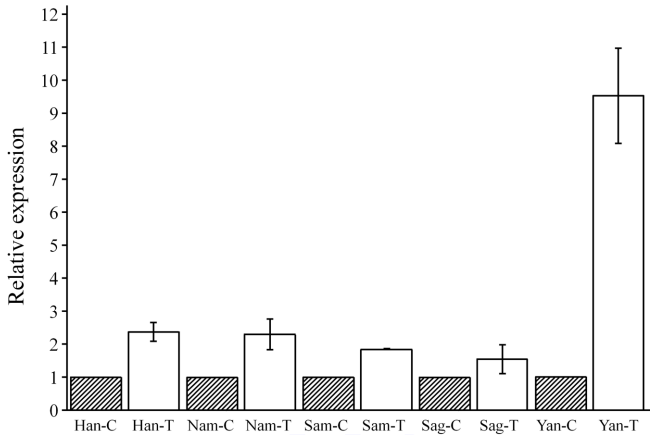

Fig. 7. Relative expression of *OsPR10b* in five resistant Korean rice cultivars after 4 days of inoculation with *Xoo* race K1.

Striped bars represent control plants and bars in white represent treated plants. Han-C: Hanareum control; Han-T: Hanareum treated; Nam-C: Namcheon control; Nam-T: Namcheon treated; Sam-C: Samdeok control; Sam-T: Samdeok treated, Sag-C: Samgang control; Sag-T: Samgang treated; Yan-C: Yangjo control; Yan-T: Yangjo treated.

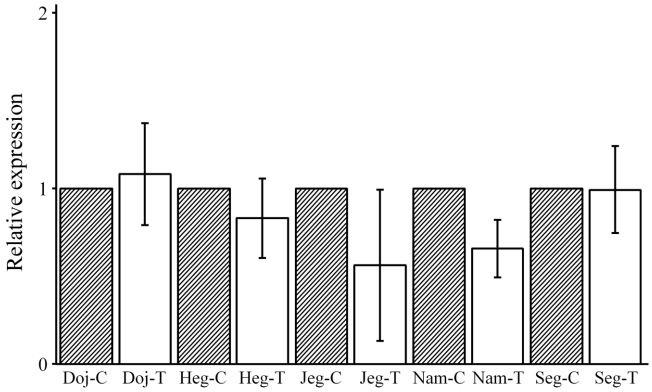

Fig. 8. Relative expression of *OsPR10b* in five susceptible Korean rice cultivars after 4 days of inoculation with *Xoo* race K1.

Striped bars represent control plants and bars in white represent treated plants. Doj-C: Dongjin1 control; Doj-T: Dongjin1 treated; Heg-C: Hegjinju control; Heg-T: Hegjinju treated; Jeg-C: Jeogtomi control; Jeg-T: Jeogtomi treated; Nam-C: Namil control; Nam-T: Namil treated; Seg-C: Seolgaeng control; Seg-T: Seolgaeng treated.

phenotype. This finding indicates that *OsPR10b* is essential for resistance to BLB. To confirm this, expression levels of *OsPR10b* were also observed in the five most susceptible cultivars (*i.e.*, Dongjin1, Hegjinju, Jeogtomi, Namil, and Seolgaeng). As a result, the expression levels of *OsPR10b* did not change significantly and were either similar (*i.e.*, Seolgaeng), lower (*i.e.*, Hegjinju, Jeogtomi, and Namil), or slightly higher (*i.e.*, Dongjin1) compared with those of the control plants (Fig. 8).

Discussion

Bacterial leaf blight of rice has been reported in several parts of the world with high incidence and severity [25]. Therefore, strategies adapted to particular environments must be developed to avoid possible epidemics. Among these many different control strategies, host-plant resistance is an important control measure. Knowledge of varietal resistance is important for selecting cultivars with durable resistance to the disease [3, 5, 21]. In this study, 32 rice cultivars were screened for resistance to *Xoo* race K1, the causal agent of BLB. Experiments were conducted in a greenhouse during winter 2014 and in the open field during summer 2015. Based on phenotypic data and visual observations of disease symptoms among the 32 rice cultivars screened, only five cultivars showed resistance to *Xoo* race K1 (*i.e.*, Hanareum, Namcheon, Samdeok, Samgang, and Yangjo) in both the greenhouse and the open field. The relatively low severity observed in the greenhouse screening compared with the open field could be due to environmental factors such as day/night temperature difference, humidity, and wind, which significantly affect the activity of *Xoo*. During their growth and development stages, plants are subjected to a wide variety of biotic and abiotic stressors. As a result, they develop defense mechanisms because of their sessile nature. During an attack from a pathogen, plants are protected by defensive signaling pathways that alert the plant [13]. Numerous genes that are essential during pathogen infection or wounding are expressed in a variety of plant species in response to pathological attacks. Five genes (*i.e.*, *JAmyb*, *OsPR10b*, *OsPR1a*, *OsNPR1*, and *OsWRKY45*) were used to identify gene expression in cultivars during screening by qRT-PCR. Among these genes, only *OsPR10b* exhibited expression levels with a positive correlation to the phenotype (Figs. 7 and 8). In resistant cultivars, *OsPR10b* expression levels were higher than in the control plants, whereas in susceptible cultivars the expression levels in treated plants were relatively similar to those in the control plants. The relatively inconsistent expression of *JAmyb* [15] in the resistant cultivars compared with control plants indicated that resistance to *Xoo* race K1 in this study is not directly through the jasmonate (JA)-signaling pathway. It is known that the activation of the JA-dependent signaling pathway is generally induced during an attack by a necrotrophic pathogen [9]. In the case of biotrophic pathogens like *Xoo*, it is normal to assume that the salicylic acid (SA)-dependent signaling pathway is triggered in order to activate a hypersensitive response [4]. However, the expression of *OsWRKY45* [22], *OsPR1a* [1], and *OsNPR1* [8, 31], which are involved in the SA-signaling pathway, also occurred in inconsistent patterns. This was especially noted with *OsNPR1*, which is a key regulator of SA-mediated resistance, where its transcript levels in all resistant cultivars were lower than those in their control counterparts. SA and JA are endogenous signal molecules required in the induction of plant immunity, in which they interact antagonistically [28-30]. The induction of *OsPR10b* in the resistant cultivar in this study might be attributed to its characteristic. In rice inoculated with the fungus *M. grisea*, the induction of *OsPR10b* took place later than that of *OsPR10a*, which started at 2 days post-inoculation [19]. Plant defense mechanisms are a result of a broad, complex, and interconnected network of genes, particularly in the interaction of rice and *Xoo* where race-specific resistance [11] and allelic interaction [17] between genes exist. Our results here provide insight and information in assessing gene expression by utilizing resistant and susceptible individuals as the test subjects. Our results reveal that some genes, including *OsNPR1*, were randomly expressed and did not show any significant correlation with the phenotype in resistant and susceptible groups; however, among the tested genes, only *OsPR10b* showed coherent expression in both resistant and susceptible individuals. Given this result, *OsPR10b* can be inferred as the gene having the most significance as a specific resistance trait to *Xoo* race K1 compared with other genes.

Acknowledgments

This work was supported by a grant from the Next-Generation BioGreen21 Program (No. PJ011257012016), Rural Development Administration, Republic of Korea. This research was supported by the Kyungpook National University Research Fund, 2013 (2014).

References

1. Agrawal GK, Jwa NS, Rakwal R. 2000. A novel rice (*Oryza sativa* L.) acidic *PR1* gene highly responsive to cut, phytohormones, and protein phosphatase inhibitors. *Biochem. Biophys. Res. Commun.* **274:** 157-165.
2. Akhtar MA, Hamed A. 2008. Comparison of methods of inoculation of *Xanthomonas oryzae* pv. *oryzae* in rice cultivar. *Pak. J. Bot.* **40:** 2171-2175.
3. Banito A, Kpémoua KE, Wydra K. 2010. Screening of rice genotypes for resistance to bacterial blight using strain × genotype interactions. *J. Plant Pathol.* **92:** 181-186.
4. Brodersen P, Malinovsky FG, Hematy K, Newman M, Mundy J. 2005. The role of salicylic acid in the induction of

cell death in *Arabidopsis acd11*. *Plant Physiol*. **138:** 1037-1045.
5. Cheema A, Awan A, Ali Y. 1998. Screening of basmati rice mutant against prevalent disease in the Punjab province. *Pak. J. Phytopathol*. **10:** 39-41.
6. Chern M, Fitzgerald HA, Canlas PE, Navarre DA, Ronald PC. 2005. Overexpression of a rice NPR1 homolog leads to constitutive activation of defense response and hypersensitivity to light. *Mol. Plant Microbe Interact*. **18**: 511-520.
7. Choi SH, Casiana MVC, Jan EL. 1998. Distribution of *Xanthomonas oryzae* pv. *oryzae* DNA modification systems in Asia. *Appl. Environ. Microbiol*. **64:** 1663-1668.
8. Feng JX, Cao L, Li J, Duan CJ, Luo XM, Le N, *et al*. 2011. Involvement of OsNPR1/NH1 in rice basal resistance to blast fungus *Magnaporthe oryzae*. *Eur. J. Plant Pathol*. **131:** 221-235.
9. Glazebrook J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol*. **43:** 205-227.
10. Gnanamanickam SS, Priyadarisini VB, Narayanan NN, Vasudevan P, Kavitha S. 1999. An overview of bacterial blight disease of rice and strategies for its management. *Curr. Sci*. **77:** 1435-1444.
11. Jeung JU, Heu SG, Shin MS, Vera Cruz CM, Jena KK. 2006. Dynamics of *Xanthomonas oryzae* pv. *oryzae* populations in Korea and their relationship to known bacterial blight resistance genes. *Phytopathology* **96:** 867-875.
12. Kauffman HE, Reddya PK, Hiesh SPY, Merca SD. 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. *Plant Dis. Rep*. **57:** 537-541.
13. Koornneef A, Pieterse CM. 2008. Cross talk in defense signaling. *Plant Physiol*. **146:** 839-844.
14. Kubo M, Purevdorj M. 2004. The future of rice production and consumption. *J. Food Distrib. Res*. **35:** 128-142.
15. Lee MW, Qi M, Yang Y. 2001. A novel jasmonic acid-inducible rice *myb* gene associates with fungal infection and host cell death. *Mol. Plant Microbe Interact*. **14:** 527-535.
16. Li ZK, Luo LJ, Mei HW, Paterson AH, Zhao XH, Zhong DB, *et al*. 1999. A "defeated" rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae*. *Mol. Gen. Genet*. **261:** 58-63.
17. Li ZK, Arif M, Zhong DB, Yu BY, Xu JL, Domingo-Rey J, *et al*. 2006. Complex genetic networks underlying the defensive system of rice (*Oryza sativa* L.) to *Xanthomonas oryzae* pv. *oryzae*. *Proc. Natl. Acad. Sci. USA* **103:** 7994-7999.
18. Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2–ΔΔCT method. *Methods* **25:** 402-408.
19. McGee DJ, Hamer JE, Hodges TK. 2001. Characterization of a *PR-10* pathogenesis-related gene family induced in rice during infection with *Magnaporthe grisea*. *Mol. Plant Microbe Interact*. **14:** 877-886.
20. Mew TW. 1984. Current status and future prospects of research on bacterial blight of rice. *Annu. Rev. Phytopathol*. **25:** 359-382.
21. Nelson RJ, Baraoidan MR, Vera Cruz CM, Yap IV, Leach JE, Mew TW, Leung H. 1994. Relationship between phylogeny and pathotype for the bacterial blight pathogen of rice. *Appl. Environ. Microbiol*. **60:** 3275-3283.
22. Shimono M, Koga H, Akagi A, Hayashi N, Goto S, Sawada M, *et al*. 2011. Rice WRKY45 plays important roles in fungal and bacterial disease resistance. *Mol. Plant Pathol*. **13:** 83-94.
23. Shimono M, Sugano S, Nakayama A, Jiang CJ, Ono K, Toki S, Takatsuji H. 2007. Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance. *Plant Cell* **19**: 2064-2076.
24. Song F, Goodman RM. 2001. Molecular biology of disease resistance in rice. *Physiol. Mol. Plant Pathol*. **59:** 1-11.
25. Sonti RV. 1998. Bacterial leaf blight of rice: new insights from molecular genetics. *Curr. Sci*. **74:** 206-212.
26. Sugano S, Jiang CJ, Miyazawa SI, Masumoto C, Yazawa K, Hayashi N, *et al*. 2010. Role of OsNPR1 in rice defense program as revealed by genome-wide expression analysis. *Plant Mol. Biol*. **74**: 549-562.
27. Swings J, van der Mooter M, Vauterin L, Hoste B, Gillis M, Mew TW, Kersters K. 1990. Reclassification of the causal agents of bacterial blight *(Xanthomonas campestris* pv. *oryzae)* and bacterial leaf streak *(Xanthomonas campestris* pv. *oryzicola)* of rice as pathovars of *Xanthomonas oryzae* (ex Ishiyama 1922) sp. nov., nom. rev. *Int. J. Syst. Bacteriol*. **40:** 309-311.
28. Tamaoki D, Seo S, Yamada S, Kano A, Miyamoto A, Shishido H, *et al*. 2013. Jasmonic acid and salicylic acid activate a common defense system in rice. *Plant Signal. Behav*. **8:** e24260.
29. Tomoya N, Mitsuhara I, Seo S, Ohtsubo N, Ohashi Y. 1998. Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. *Plant Cell Physiol.* **39:** 500-507.
30. Van der Does D, Leon-Reyes A, Koornneef A, Van Verk MC, Rodenburg N, Pauwels L, *et al*. 2013. Salicylic acid suppresses jasmonic acid signaling downstream of SCFCOI1-JAZ by targeting GCC promoter motifs via transcription factor ORA59. *Plant Cell* **25:** 744-761.
31. Yuan Y, Zhong S, Li Q, Zhu Z, Lou Y, Wang L, *et al*. 2007. Functional analysis of rice *NPR1*-like genes reveals that *OsNPR1/NH1* is the rice orthologue conferring disease resistance with enhanced herbivore susceptibility. *Plant Biotechnol. J*. **5:** 313-324.