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Full Length Article

Screening Rice (*Oryza sativa*) Cultivars for Resistance to Bacterial Leaf Blight Disease

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Abstract

Bacterial Leaf Blight (BLB), caused by *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*), is a devastating disease that affects the yield and quality of rice (*Oryza sativa* L.) produced throughout the world. However, resistant rice cultivars do exist, and utilizing them in regions impacted by BLB is an important tool in reducing crop loss. In this study, six rice cultivars widely grown in Ghana along with controls were phenotypically screened for resistance to *Xoo* strain K1 under greenhouse conditions. Phenotypic assessment identified the rice cultivar Popa as the most resistant Ghanaian phenotype. To investigate cultivars carrying *Xanthomonas* resistance alleles (*Xa-R* genes), five STS/SSR markers (*RM-317, RM-224, RM-13, xa-13prom* and *pTA248*) respectively linked to *Xa2, Xa4, xa5, xa13* and *Xa21* were used to genotype selected cultivars. Our results revealed that Ghanaian cultivars, Tinsibe, AGRIC-1 and Krampa White, carry *Xa2*; Kabre and Krampa White carry *Xa4*; and Popa and IRAT10 carry *xa5*. However, none of the 10 cultivars showed the presence of *xa13* and *Xa21*. The *O. sativa* subsp. *indica* resistant control, Tetep, contained *Xa2* and *xa5*, whereas the susceptible control, IR661, contained *xa5*. Quantitative real-time PCR (qRT) results of selected cultivars revealed variable expression profiles of *OsWRKY45, OsPR10b, OsJAZ8* and *OsPR1a* in response to *Xoo* infection. Though one or more genes responsible for *Xoo* resistance were present in Ghanaian cultivars, most still exhibited a susceptible phenotype following *Xoo* infection, which indicates that these *Xa* genes identified primarily from East Asian germplasm that typically confer resistance are not the primary source of resistance in West African rice cultivars to the *Xoo* K1 strain.

Keywords: Bacterial leaf blight; *Xoo* K1 strain; STS/SSR markers; Disease resistance; Rice

Introduction

Rice (*Oryza sativa* L.) accounts for 1/5 of all calories consumed by people, and is the third most economically important crop in the world (Izawa and Shimamoto 1996). Rice production is essential in many economies, especially developing economies, such as Ghana. The BLB disease (BLB), caused by *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*), is a destructive disease that negatively impacts rice yield throughout the world. Bacterial leaf blight disease has been extensively studied in Asia, especially compared to Africa where *Xoo* shows high pathogenic variability (Séré *et al.* 2013). The first BLB disease incidence reported in many African countries occurred in the 1980s, particularly in West African rice growing regions (Wonni *et al.* 2014). This disease is considered an emerging disease in West Africa and other parts of the African Continent, and can cause significant crop loss.

This disease is characterized by a continuous reduction in the yield and quality of the crop. According to Mew *et al*. (1993), yield reduction due to a mild BLB infection is about 10–20%, whereas a severely infected rice field may exhibit a 50% crop loss. During epidemics, yield losses as high as 80% have been recorded. The severity of crop infection and resulting crop loss is dependent on the *Xoo* strain and the rice variety, growth stage, geographical location and seasonal conditions (Wonni *et al.* 2016). Infection occurs when bacteria (*Xoo*) penetrate infection courts (leaves) through wounds or hydathodes, and then spread through the leaf and colonize xylem vessels. There are several identified pathovars, and they defeat the plant defense system and induce a set of diverse pathways that ultimately result in a successful infection and bacterial leaf blight disease.

Primarily, seedlings and adult plants are infected by the *Xoo* pathogen, resulting in wilting of young plants and leaf blight symptoms, a syndrome referred to as kresek

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(Wonni *et al.* 2014). Infected, symptomatic plants also display pale yellow leaves. Symptoms are typically observed in young plants of susceptible cultivars in the tropics at tillering stage. At this infection stage, the infected plants may roll and wilt as well as turn grey-green. Entire plants may even eventually die (Wu *et al.* 2011). Bacterial leaf blight disease incidence has increased recently most likely owing to the use of intensive agronomic practices that generate conditions favorable to the development of this disease, such as high rates of nitrogen fertilizers, close spacing and continuous cropping with susceptible cultivars (Gale *et al.* 1985).

Antibiotic development and use to control such a pathogen on a commercial, agricultural scale is unlikely and impractical (Gnanamanickam *et al.* 1999). Therefore, the most reliable method of control is to use genetically resistant cultivars. Several cultivars containing known resistant loci have been identified (Zheng *et al.* 2009). Continued identification of diverse BLB resistant and susceptible cultivars using conventional screening techniques is hindered by environmental effects. However, known, environmentally independent molecular markers associated with foliar disease resistance have accelerated the identification processes of resistant genotypes. To date, more than 38 loci have been identified as conferring strong resistance against different strains of *Xoo* in rice (Jeung *et al.* 2006; Niño-Liu *et al.* 2006; Pradhan *et al.* 2015; Dilla-Ermita *et al.* 2017; Nguyen *et al.* 2018), which are referred to as resistance or '*R*' genes.

The recent, exponential progress in rice genomics research and the successful completion of sequencing the rice genome is allowing researchers to precisely identify many agronomically important genes. To date, four of the identified *Xa*-*R* genes have been cloned and extensively studied (Blair and McCouch 1997; Iyer and McCouch 2004, 2007; Salgotra *et al.* 2011; Singh *et al.* 2015). *R* gene *Xa1* confers resistance to race 1 isolates of *Xoo* in Japan and *Xa2* to the Japanese *Xoo* strain T7147 (Sakaguchi 1967). *Xa4* confers durable resistance in Asian rice (Mew *et al.* 1992). *xa5* (Iyer and McCouch 2004) and *xa13* (Chu *et al.* 2006; Antony *et al.* 2010) are recessive *R* genes that only confer resistance when they are present in their homozygous state, whereas *Xa2*1 is a dominant *R* gene that confers broad spectrum resistance against *Xoo* strains belonging to different races of the pathogen (Song *et al.* 1995).

Many practical markers for tagging and marker assisted selection are microsatellites since they are co-dominant, PCR-based, and can detect high levels of polymorphism. Several DNA marker types, including microsatellites, have been used to investigate rice cultivars carrying BLB resistance genes, and rice breeding programs benefit from the identification of resistant germplasm and resistance genes. Therefore, the aim of this study was to screen different rice ecotype cultivars originating from Ghana for varietal resistance to BLB disease. The specific objectives were to: (1) assess the phenotypic response of 10 rice cultivars following BLB inoculation, (2) conduct a genotyping-based assessment to determine the presence or absence of resistance alleles *Xa2, Xa4, xa5, xa13* and *Xa21* using polymerase chain reaction (PCR) -based molecular markers (STS and SSR), and (3) investigate the expression of the plant defense-related genes, *OsWKY45, OsJAZ8, OsPR1a* and *OsPR10b* among selected rice cultivars.

Materials and Methods

Materials

Ten rice genotypes, six of which are local rice accessions from Ghana, including Popa Tos13150, IRAT 10, Kabre, Tinsibe, AGRIC-1 and Krampa White, and four resistant and susceptible controls, were used to conduct this study (Table 1). Cultivars Tetep (Blair and McCouch 1997) and Jinbeak (Kim *et al.* 2009) served as resistant controls, whereas Nampyeong (Fred *et al.* 2016) and IR661 served as susceptible controls to *Xoo* K1 strain. All of the Ghanaian cultivars as well as Tetep and IR661were *O. sativa* subsp. *indica*, whereas as Jinbeak and Nampyeong were *O. sativa* subsp. *japonica.* Samples of all 10 rice cultivars were provided by the National Agro-biodiversity Center (NAC) in Jeonju, Republic of Korea.

Experimental design and growth conditions

This study was conducted under greenhouse conditions at Kyungpook National University, Daegu, Republic of Korea. The experiment was a complete randomized design with three replicates. Seeds of each of the 10 cultivars were sown in Petri dishes, incubated and germinated at ± 25°C for two weeks. Approximately 14 day-old seedlings were then transplanted into 50 cm diameter plastic pots and kept under greenhouse conditions. Plants were grown under a 16 h/8 h light and dark cycle at a temperature ranging between 25 and 30°C in the greenhouse.

Pathogen growth and inoculation to plants

The *Xoo*, K1 strain (K1 race) is a Korean strain of *X. o.* pv. *Oryzae*, and was obtained from the National Agrobiodiversity Center in Jeonju, Republic of Korea. Bacterial cultures were grown and incubated on potato sucrose agar (PSA) Petri plates prepared using 5 g Bacto-peptone (Becton, USA), 0.5 g sodium L-glutamate monohydrate, 5 g sucrose and 8 g Bacto-agar at 30°C overnight. Single colonies were picked and grown on PSA medium at 30°C overnight. Bacterial counts were then adjusted to 0.002 CFU/mL by measuring the optical density of the culture at 600 nm using a spectrophotometer as described previously (Yin *et al.* 2017).

Three replicates of fully expanded leaves of well-acclimatized plants were inoculated with *Xoo* culture 40

days after germination. Three leaves per plant were inoculated through the leaf clipping method (Kauffman 1973). A 2 cm piece from each leaf tip was clipped using a sterile scissor and dipped into the bacterial solution (0.002 CFU/mL). Negative controls were mock inoculated using only sterile distilled water. Plants were kept at 35 ± 2°C under greenhouse conditions, and symptoms development was closely monitored.

Measurement of disease severity and pathogenicity assessment

After inoculation, leaf samples were collected at three set time points, 4, 10, and 14 days' post inoculation (dpi), in order to observe the response of inoculated cultivars to *Xoo* inoculation. To confirm and evaluate *Xoo* infection, leaf extract was spread on PSA medium with cephalexin, and colony counts were recorded (Wang *et al.* 1996). The identification of *Xoo* specific symptoms was performed based on morphological characteristics previously described (Swings *et al.* 1990) and further confirmed through 16srRNA sequencing (Zhang *et al.* 2000). The sequencing results are reported in Fig. S1.

Disease severity was scored using a previously described disease rating scale (Gnanamanickam *et al.* 1999; Waheed *et al.* 2009). Scoring was performed 14 dpi. Disease symptoms were recorded from the leaf tip to the base of the blade (Gourieroux *et al.* 2017). The lesion size percentage was recorded using the equation below as described by Kauffman (1973).

$$\text{Lesion size (Percentage of leaf length)} = \frac{Lesion\ length}{Total\ leaf\ length} \times 100$$

The BLB disease severity scoring was classified using a disease index scale listed in Table 2 (Chaudhary 1996).

STS/SSR markers analysis

Approximately 20-day old leaves were collected from each cultivar for STS/SSR marker analysis. DNA was extracted using the CTAB method as described by Goto *et al.* (1999). The concentration and quality were checked using NanoQ (Optizen, South Korea). Four previously reported SSR markers and one STS marker (*RM-317*, *RM-224, RM-13, xa-13prom* and *pTA248*, respectively) were used to screen 10 rice cultivars for the absence or presence of five common BLB resistance loci linked to *Xa2, Xa4, xa5, xa13 and Xa21 R* genes, respectively (Singh *et al.* 2015). There are 40+ known *Xa-R* genes, and these were chosen due to the fact that they tend to confer broader resistance as opposed to race-specific resistance.

A 20 μL reaction mixture, including 2X F-Star Taq PCR Master mix (BioFact™, South Korea) and 10 μM of each marker specific forward and reverse primers, was used to amplify the selected DNA markers (Applied Biosystems, California, U.S.A.). Additional information on the markers used is provided in Table S1. The PCR conditions were as follows: initial polymerase activation at 94.0°C for 2 min followed by 35 cycles of 94.0ºC for 15 s, 58.5ºC–61.4ºC for 30 s (optimized individually for each marker primer) and 72.0ºC for 1 min 30 s with a final extension of 72.0ºC for 5 min. Amplified PCR products were analyzed using gel electrophoresis with a 3% agarose gel and visualized with a gel documentation system (Uvitec Cambridge, UK).

Table 1: Characteristics and source of collection of rice genotypes used for the studies

Rice Varieties	Subspecies	Country of Origin	Source
Tetep	*Indica*	China	NAC- South Korea
IR661	*Indica*	Philippines	NAC- South Korea
Jinbeak	*Japonica*	Korea	NAC- South Korea
Nampyeong	*Japonica*	Korea	NAC- South Korea
Popa Tos 13150	*Indica*	Ghana	NAC- South Korea
IRAT 10	*Indica*	Ghana	NAC- South Korea
Kabre	*Indica*	Ghana	NAC- South Korea
Tinsibe	*Indica*	Ghana	NAC- South Korea
AGRIC -1	*Indica*	Ghana	NAC- South Korea
Krampa White	*Indica*	Ghana	NAC- South Korea

Table 2: BLB disease severity and evaluation scale

Disease rating	Lesion size (% of leaf length)	Interpretation
0	0	Immune (I)
1	>1-10 %	Resistant (R)
3	>11-30 %	Moderate Resistant (MR)
5	>31-50 %	Moderately Susceptible (MS)
7	>51-75 %	Susceptible (S)
9	>76-100 %	Highly Susceptible (HS)

Source: (Chaudhary 1996)

Quantitative real-time PCR (qRT-PCR) analysis

RNA extraction and qRT-PCR were performed as described in Imran *et al.* (2018). Briefly, total RNA was extracted using the TRIzol® reagent method. The quality and quantity of RNA were checked with agarose gel electrophoresis and NanoQ (OPTIZEN, South Korea), respectively. Complementary DNA (cDNA) was synthesized as described by Imran *et al.* (2018). A two-step real-time PCR reaction was performed using an Eco ™ real-time PCR system (Illumina, California, U.S.A.) using 2x Real-Time PCR Master mix including SYBR Green I (BIOFACT, South Korea) with 100 ng of template DNA and 10 n*M* of each primer in a final volume of 20 μL. The PCR conditions were polymerase activation at 95°C for 15 minutes and concurrent denaturation at 95°C, annealing and extension at 60°C for 40 s for a total 40 cycles. The primer list is given in Table S2.

Statistical analysis

GraphPad Prism 7.03 (GraphPad, California, U.S.A.) was used to perform an analysis of variance (ANOVA) on the experimental data. Means were separated using least significant difference (LSD) at a 5% probability level. DNA banding profiles were recorded as present or absent.

Table 3: Marker results of 10 accessions screened for BLB resistance. Cultivars are listed based on lesion % for both the control and Ghanaian cultivars from lowest to highest. Positive (+) and negative (-) signs indicate the presence and absence of Xa R genes in a particular cultivar, respectively

Category	Rice Subspecies	Accession Number	Local Name	BLB resistance genes				
				Xa2	*Xa4*	*xa5*	*xa13*	*Xa21*
Controls								
Resistant	*japonica*		Jinbeak	-	-	+	-	-
Resistant	*indica*	IT 102103	Tetep	+	-	+	-	-
Susceptible	*indica*	IT 001944	IR661	-	-	+	-	-
Susceptible	*japonica*		Nampyeong	-	+	-	-	-
Ghanaian cultivars								
Resistant	*indica*	IT 226965	Popa	-	-	+	-	-
Susceptible	*indica*	IT 283479	Krampa White	+	+	-	-	-
Susceptible	*indica*	IT 226946	Kabre	-	+	-	-	-
Susceptible	*indica*	IT 267919	AGRIC -1	+	-	-	-	-
Susceptible	*indica*	IT 214850	IRAT 10	-	-	+	-	-
Highly susceptible	*indica*	IT 226964	Tinsibe	+	-	+	-	-
Approx. size (bp)				154	160	139	498	982

Resistance and susceptibility to *Xoo* strain K1

Relative expression levels were determined by comparing treated and control plants both normalized to *OsUBI*.

Results

Genotypic screening for BLB resistance locus *Xa*

The presence or absence of resistant locus *Xa* was evaluated in all cultivars through marker genotyping and is shown in Table 3. Tested cultivars received a negative score if no *Xa R* gene was present for each corresponding SSR or STS marker, a non-specific band was amplified, or a band size corresponding to susceptible genotypes was present. When the presence of an expected size SSR or STS band was present, cultivars were scored positive. The genotyping results revealed that Ghanaian cultivars, Tinsibe, AGRIC-1 and Krampa White, carry *Xa2*; Kabre and Krampa White carry *Xa4*; and Popa and IRAT10 carry *xa5*. However, none of the 10 cultivars showed the presence of *xa13* and *Xa21*(Fig. S2; Table 3). Tetep, the *O. sativa* subsp. *indica* resistant control harbors *Xa2* and *xa5*, and the susceptible IR661 only carried *xa5*. Interestingly, Jinbaek, the *O. sativa* subsp. *japonica* resistant control, only harbored *xa5* out of the five BLB *R* genes screened for as well, while the susceptible Nampyeong carries *Xa4*.

Symptoms development in selected rice cultivars to *Xoo* bacterial inoculation

All of the 10 cultivars mentioned in Table 1 were screened in a pilot experiment, particularly for their response to *Xoo* inoculation. Based on the initial screening results (Fig. S3), five cultivars were selected, which included Tetep, Jinbaek, Popa, Tinsibe and AGRIC-1, for further testing. The results suggest that the Ghanaian cultivar Tinsibe was the most susceptible cultivar tested, and it showed severe symptom development (Fig. 1A) followed by AGRIC-1. The known South Korean cultivar Jinbaek showed the most resistance to *Xoo* infection followed by Tetep (Fig. 1A). The Ghanaian cultivar Popa also showed resistance to *Xoo* K1 strain, and a similar response was observed in Tetep (Fig. 1A).

The initial symptoms that appeared as colonies were circular, convex and light yellow that looked smooth. It took 3–5 dpi for the yellow pigment on the leaf to appear in the form of a curl. Symptom development was further quantified by measuring lesion length. The disease score was calculated as the percentage of the lesion length of each cultivar relative to the total leaf length. Jinbaek was observed to be the most resistant cultivar and exhibited a shorter lesion length at all time-points (Fig. 1A), whereas Tinsibe was the most susceptible, showing significantly longer lesion length compared to other cultivars (Fig. 1A). At 4 dpi, there was no significant difference in lesion length among cultivars. However, at 10 dpi, the invasion of the pathogen accelerated and was traceable through the development of symptoms in susceptible genotypes. Jinbaek had a mean lesion length of 0.29 cm, while Tinsibe had one of 4.33 cm (Fig. 1B). Similarly, at 14 dpi Tinsibe showed the longest mean lesion length (8.52 cm), and Jinbaek showed the shortest mean lesion length (0.41 cm). Among all Ghanaian cultivars, Popa was the most resistant with a mean 0.9 cm lesion length at 14 dpi compared to Tinsibe and AGRIC-1 having 7.8 and 4.2 cm mean lesion lengths, respectively (Fig. 1B).

Pathogenicity assessment of *Xoo*

To assess the pathogenicity of *Xoo* K1 strain in different cultivars, the disease severity percentage was calculated to give a better understanding of the individual varietial responses to *Xoo* K1 infection. The results showed that Tetep (4.9%), Jinbeak (1.4%) and Popa (3.1%) had the lowest disease severities (percentage of leaf length affected), and were subsequently classified as resistant based on the standard scoring proposed by Chaudhary (1996). Moderate susceptibility was observed in AGRIC-1 (31.5%), whereas Tinsibe (77.9%) was highly susceptible (Fig. 2).

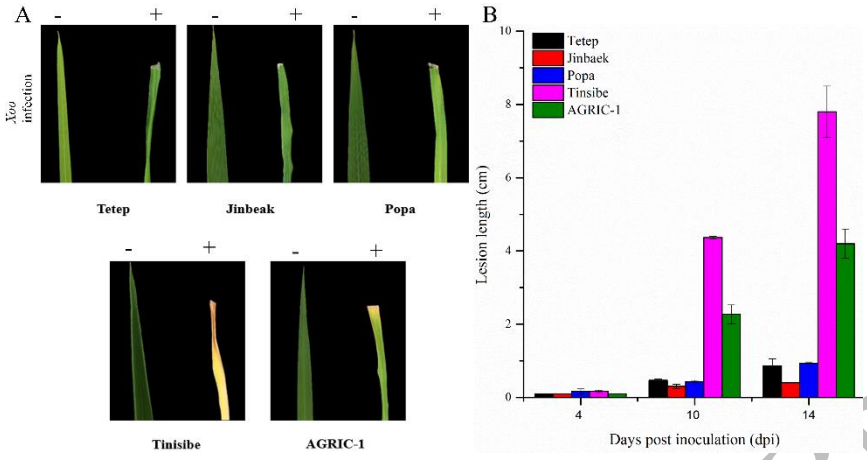

Fig. 1: Response of different rice cultivars towards attempted *Xoo* infection. (**A**) Symptom development after 14 dpi. The leaves on the right are inoculated with *Xoo*, whereas those on the left were mock inoculations only containing sterile water. (**B**) Quantification of lesion length in cm of select cultivars. Each data point is the mean of three replicates. Error bars indicates means ± S.E. (n = 3)

Gene expression (transcript levels) of pathogenesis-related genes

To determine whether disease severity or resistance is related to pathogenesis-related (PR) genes, the expression of known PR genes was evaluated in the selected cultivars 4 dpi with *Xoo*. This time point was selected since it was when the cultivars typically started to show symptoms of *Xoo* infection (Fig. 1). The results showed that a jasmonic acid (JA)-mediated BLB resistant gene, *OsJAZ8*, was up-regulated in Jinbaek and AGRIC-1 and down-regulated in Popa and Tinsibe compared to control plants (Fig. 3A). Tetep did not show any significant difference in *OsJAZ8* gene expression (Fig. 3A). Similarly, the expression of *OsWRKY45*, another disease-related gene, was up-regulated only in AGRIC-1 and down-regulated in all other tested cultivars (Fig. 3B). Furthermore, the expression of salicylic acid (SA)-pathway related genes, *OsPR1a* and *OsPR10b*, were evaluated, and the results showed that Jinbaek and Tinsibe have increased transcript accumulation of *OsPR1a* and the other cultivars had a decrease in expression level compared to control plants (Fig. 3C). Transcript accumulation of *OsPR10b* was highest in Tetep followed by Tinsibe and Jinbaek and down-regulated in Popa (Fig. 3D).

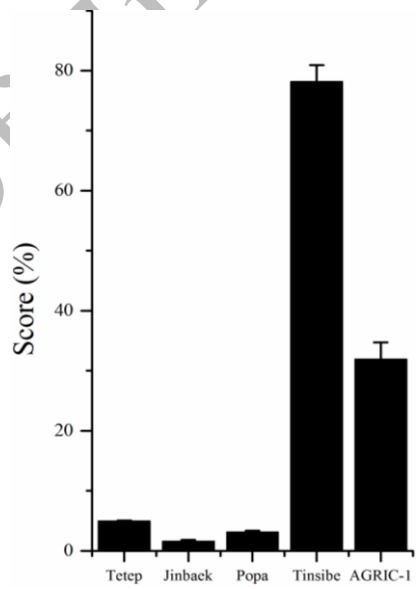

Fig. 2: Disease severity scoring by percentage of five representative cultivars. The scale rating used based on Chaudhary (1996). All the data points represent the mean of three replicates. Error bars represent ± SE

Early transcript accumulation of *OsWRKY45* and *OsPR10b* in selected cultivars

To understand the early response of two important biotic stress related genes, *OsWRKY45* and *OsPR10b*, to *Xoo* infection, three cultivars, Tetep (resistant control *indica*), Tinsibe (susceptible) and Jinbaek (resistant control *japonica*), were selected and inoculated with *Xoo* K1 strain. Gene expression was measured at 0, 6, 12, 24 and 48 h post *Xoo* infection. The expression of *OsPR10b* was up-regulated only in the resistant cultivars Tetep and Jinbeak, particularly at 12 h post inoculation, whereas it was down-regulated in the susceptible cultivar Tinsibe. These findings indicate that *OsPR10b* is positively correlated with plant resistance against *Xoo* and that the resistance of Tetep and Jinbaek may be due to an early increase in the expression of *OsPR10b* (Fig. 4A). Furthermore, the expression of *OsWRKY45* was down-regulated in response to *Xoo* inoculation in all cultivars at all-time points (Fig. 4B).

Discussion

Verdier *et al.* (2012) hypothesized that African wild rice germplasm likely contains novel resistance loci and can

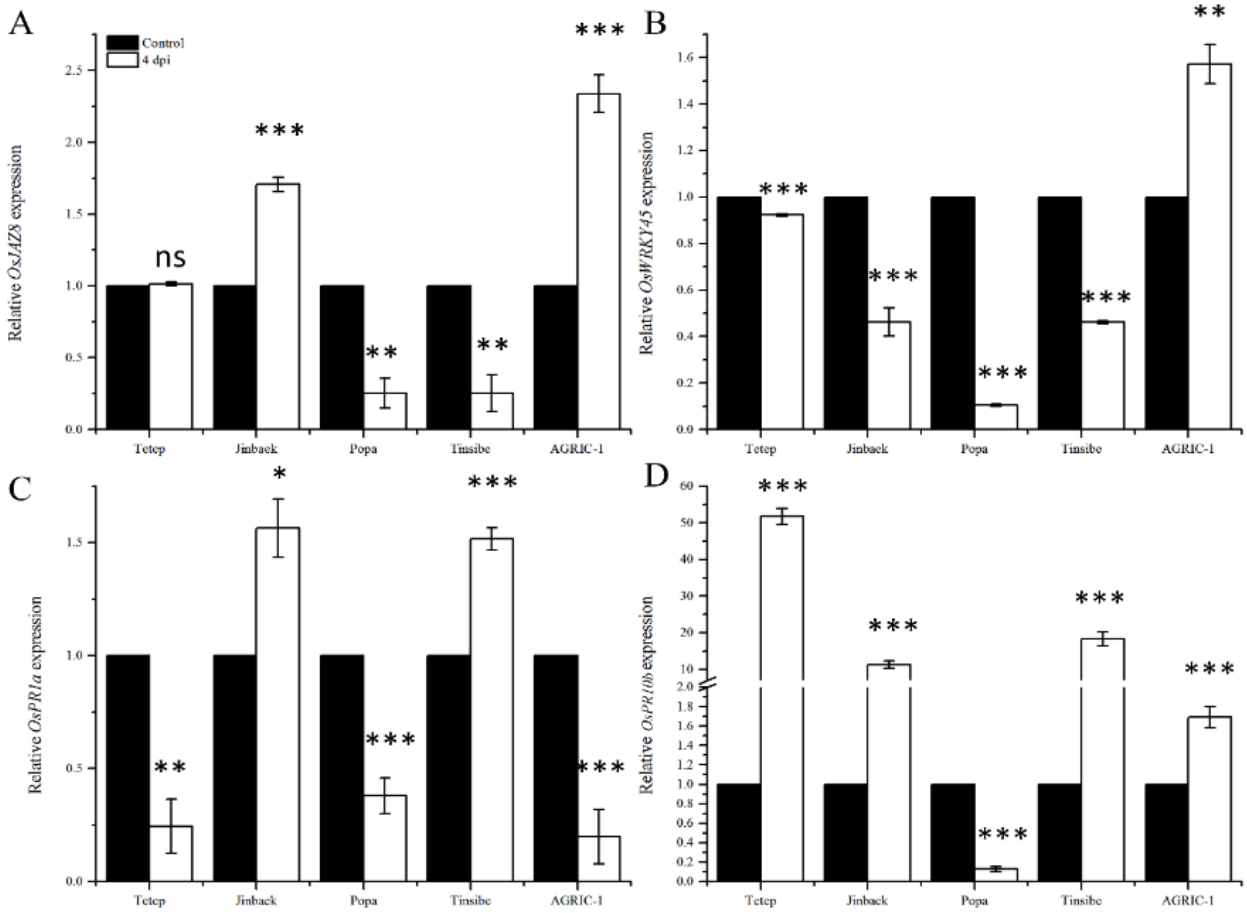

Fig. 3: Relative expression of selected pathogenesis-related genes after 4 dpi

Relative expression of (**A**) *OsJAZ8* (**B**) *OsWRKY45* (**C**) *OsPR1a* and (**D**) *OsPR10b*. Each data point represents the mean of three replicates. Black and white bars indicate the expression levels of the same genes in control and treated plants, respectively. Error bars indicate ± SE. Asterisks represent significant differences in relative expression of genes in *Xoo* treated and control plants (ns= non-significant, *=$P < 0.05$, **=$P < 0.01$, ***=$P < 0.001$)

serve as valuable genetic resources for identifying and deploying new *R* genes. To better understand the available resistance in Ghanaian rice germplasm, six West African rice cultivars were evaluated first phenotypically for their response to the Korean *Xoo* K1 strain and then genotyped with five of the more common *Xa-R gene* markers. In the current study, three of the Ghanaian rice cultivars, Krampa White, Tinsibe and AGRIC-1, harbored the *Xa2 R* gene like the resistant control Tetep. However, while Krampa White and Tetep had a resistant phenotype, Tinsibe and AGRIC-1 were susceptible to *Xoo* K1 strain. While *Xa2* may function as and *R* gene in Asian cultivars, this inconsistency in phenotypic response to *Xoo* infection in the West African cultivars evaluated suggests that it does not function in the same way in that genetic background.

The *Xa4* gene is reportedly one of the most widely studied resistance genes in many Asian rice breeding programs, and it confers durable resistance in many commercial rice cultivars (Mew *et al.* 1993). It has been mapped on to chromosome 11 with a linkage distance of 1.0 cM to the widely used, reliable RM-224 marker (Sun *et al.* 2003). Our results revealed the presence of *Xa4* in two resistant Ghanaian cultivars, Kabre and Krampa White, and in the Korean susceptible, control *japonica* cultivar Nampyeong. While Nampyeong showed mild susceptibility to *Xoo*, the African cultivars Kabre and Krampa White may owe their resistance status to the presence of *Xa4*, and it could be of use in future BLB resistance breeding. However, Kabre and Krampa White showed some susceptibility during initial screening. This inconsistency may be due to a masking effect of other genes in these cultivars or any number of epistatic interactions, reducing the expression of their resistance. It could also be due to the virulent nature of the K1 *Xoo* strain used in our study, and *Xa4* may not impart complete resistance to the *Xoo* K1 race in these cultivars. Still, *Xa4* may confer resistance to other *Xoo* races.

The SSR marker RM-13 was used to screen for resistant gene *xa5*, which maps at a linkage distance of 17.9 cM (Blair and McCouch 1997). This potential resistance gene is frequently present in *indica* cultivars. Blair and McCouch (1997) studied microsatellites and sequence-tagged sites diagnostic for the rice BLB resistance gene *xa5*

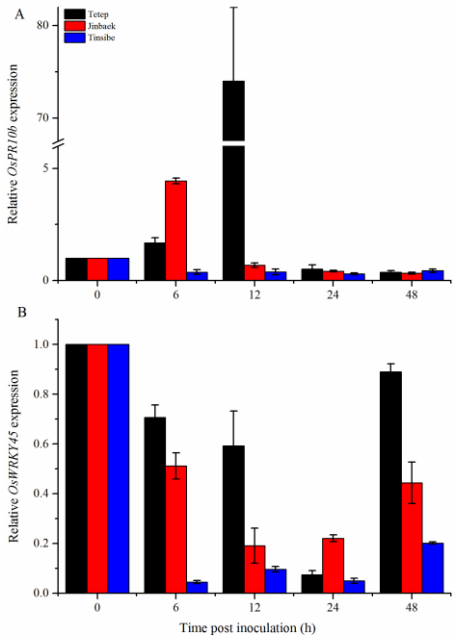

Fig. 4: Early transcript accumulation at 0, 6, 12, 24, and 48 h after infection with *Xoo* of (**A**) *OsPR10b* and (**B**) *OsWRKY45*. Plants of three selected cultivars were inoculated with *Xoo* and the expression of the indicated genes was measured overtime relative to *OsUBI* expression through qRT-PCR. Values are means ± SE of three replicates. Five leaf blades were pooled to make one replicate with three replicates in total

in 122 rice accessions, and they found that for all of the genotypes evaluated, *xa5* donor and recurrent parents had *indica* backgrounds. *xa5* was absent from all of the *japonica* genotypes from South Korea. Furthermore, Busto *et al*. (1990) showed that the *xa5* gene is more pronounced among isozyme group II, which is a distinct group derived from the *indica* subspecies. This study suggested that the center of origin of *xa5* is likely the Indian subcontinent (Nepal, Pakistan, India and Bangladesh), especially given that *xa5* has not been found in any varieties from South Korea, Japan, Taiwan or the Philippians (Busto *et al.* 1990). In the current study, this marker was identified in the West African cultivars Popa, IRAT 10 and Tinsibe, as well as both the resistant and susceptible *indica* controls Tetep and IR661. Since the marker occurred in the most resistant and susceptible cultivars tested, *xa5* likely does not play a role in the resistance phenotype to *Xoo* K1 strain.

xa13 gene is fully recessive, conferring resistance only in the homozygous state (Khush and Angeles 1999; Chu *et al.* 2006). Gene expression studies using pathogen-induced subtractive cDNA library analysis have revealed that some defense responsive genes activated in *xa13*-mediated resistance are not controlled by dominant *R* genes (Wen *et al.* 2003). For the resistance to detectable, the cultivars tested would have needed to be in a homozygous state. Unfortunately, the evaluation of the presence of the resistance gene *xa13*, which maps at a distance of 3.7 cM, resulted in a single band approximately 280 bp in all the rice cultivars studied (Fig. S2). No polymorphism was detected in any of the cultivars, which indicates that *xa13* was absent or it could not be detected by the xa-13prom (SSR) due to some recombination in the 3.7 cM region between the *xa13* locus and marker.

In addition, no amplicon specifically visualized to *Xa21*, which encodes a leucine-rich repeat receptor-like kinase, were detected for any of the 10 rice cultivars evaluated. Therefore, both *xa13* and *Xa21* were determined to be absent from all the accessions evaluated. Similar results were also reported on Indian rice genotypes used in other breeding programs (Davierwala *et al.* 2001; Singh *et al.* 2013). Meanwhile, Song *et al*. (1995) showed that rice cultivars carrying *Xa21* are able to induce an effective defense response to multiple strains of the bacterial *Xoo* pathogen. Moreover, many genes that are required for *Xa21* gene activation mediated immunity have been identified in *Xoo* (Shen *et al*. 2002).

Out of the six Ghanaian local cultivars, only two of them harbored more than one of the resistance genes screened for. Tinsibe possessed *Xa2* and *xa5*, and Krampa White harbored *Xa2* and *Xa4*. Interstingly, Gonzalez *et al.* (2007) reported that *Xa2* and *Xa4* have race-specific resistances to African *Xoo* strains. These observations are similar to those reported by Ullah *et al*. (2012) that showed that out of 52 materials, only 10 basmati rice landraces had multiple resistance genes. All Ghanaian cultivars harbored one or two genes (*Xa2*, *Xa4*, and *xa5*), and the presence of *Xa-R* genes already in local Ghanaian backgrounds, either individually or in combination, may be useful for providing resistance against African strains of *Xoo* (Verdier *et al.* 2012).

The most resistant control, Jinbeak, possessed *xa5* (Table 3). One study regarding the response of Jinbaek to BLB reported that this rice cultivar exhibited resistance against *Xoo* K1, K2, K3, and K3a infection (Kim *et al.* 2009). The authors also found that Jinbaek carries *Xa3* in addition to *xa5*. Therefore, the reported resistance phenotypic response of Jinbaek to K1 infection in the current study supports that *xa5* may have some race specificity to K1 strains, which likely contributes to much of its resistance to BLB.

These findings indicated that local African cultivars as well as other landrace cultivars conserved by farmers show potential for discovering previously unknown resistant lines useful in future breeding programs. Many cultivars showed the presence of one or more genes responsible for *Xoo* resistance. However, most of the cultivars were found to be susceptible to *Xoo* K1 strain infection indicating that these *Xa-R* genes may be non-functional or race specific. Screening these cultivars against *Xoo* strains occurring in Western Africa would better determine the level of field resistance these cultivars have.

Based on initial screening, five cultivars were selected to be evaluated for their response to *Xoo*. Similar disease

and symptom development observations consistent with those of this study were made by Kauffman (1973) and Noor *et al.* (2006) who reported that the *Xoo* appearance occurs within 4–5 dpi in the form of leaf curling. None of the cultivars were found without lesions at 14 dpi, which indicates that none of the genotypes were completely resistant or immune to *Xoo*. This also validates that the K1 strain of *Xoo* used in this study was virulent.

In addition, the resistant cultivars, Jinbaek, Tetep and Popa, showed symptom development at a later stage (*i.e.*, 11 dpi), which suggests that the pathogen is able to infect and start causing damage in susceptible cultivars earlier, whereas relatively resistant varieties seem to initially inhibit the bacteria from causing infection or disease. In a similar study, Singh *et al*. (2013) reported that the first symptoms of BLB appeared 7 dpi in moderately susceptible cultivars. AGRIC-1 showed moderate susceptibility, which is consistent with the findings by Agaba *et al.* (2015). The Ghanaian cultivar Popa shows phenotypic resistance to BLB at 14 dpi, making it a good candidate for inclusion in BLB resistance breeding programs.

Plants have evolved disease resistance in response to pathogen attack by activating systems controlled through various signaling pathways. Diverse regulatory pathways have been identified and are possible targets for plant genetic manipulation for disease resistance. Both JA- and SA-signaling pathways have been identified for the use of mediating responses against these infectious diseases in plants. The JA-induced *OsJAZ8* was up-regulated in AGRIC-1 and Jinbaek, both cultivars at least somewhat resistant to BLB, whereas it was down-regulated in the susceptible cultivar Tinsibe (Fig. 3A). The induced expression of *OsJAZ8* may be due to the inoculation method that consists of cutting the leaf that induced a wound response, a JA-related process. The inclusion of healthy, uninoculated controls in future studies will assist in determining if inoculation method is a factor affecting expression. Conversely, in the well-studied resistant cultivar Jinbaek, a reduction in the expression of *OsWRKY45* was observed, which may be due to the negative regulation of disease by *OsWRKY45* consistent with that described by Huangfu *et al.* (2016). Other reports have confirmed that SA-mediated plant defense signaling pathways are present in rice (Silverman *et al.* 1995; Yang *et al.* 2004).

Pathogenesis-related proteins, whose production and accumulation have been reported to be a vital component of the active plant defense repertoire, are increasingly studied as important factors in disease resistance (Agrawal *et al.* 2001). Results of the current study showed increased transcript accumulation of *OsPR1a* in Jinbaek and Tinsibe and of *OsPR10b* in Tetep followed by Tinsibe (Fig. 3C, D). *OsPR10b* was likely mainly responsible for the SA-induced PR gene expression owing to the fact that the transcript accumulation of *OsPR10b* was almost 10 times higher than that of *OsPR1a* expression. This hypothesis is also consistent with other studies (Jwa *et al.* 2001). The increased expression of PR-related genes in Tinsibe was unexpected and inconsistent with the observed susceptible phenotypic response of this cultivar. However, this increased *OsPR10b* accumulation may be due to delayed induced expression.

Fred *et al*. (2016) conducted a comprehensive screening of various rice genotypes for BLB resistance to Korean K1 strain, and the findings reported showed that the phenotype recorded during the experiment and the expression patterns of *OsNPR1*, *OsPR1a*, *OsWRKY45*, *etc*. were not well correlated. The transcript accumulation of these genes was also found to be unexpectedly down-regulated in resistant cultivars leading Fred *et al*. (2016) to conclude that *OsPR10b* was the only defense-related gene with a coherent transcriptional pattern correlated with the observed phenotype in resistant and susceptible genotypes. Given findings from previous studies, the early response of susceptible and resistant cultivars to *Xoo* was determined by studying *OsPR10b* and *OsWRKY45* expression (Fig. 4A, B). It was confirmed that Tinsibe showed a reduction in transcript accumulation of *OsPR10b* overtime, while Tetep and Jinbaek showed an increase in transcript accumulation at early time points (Fig. 4A). This indicates that PR-related gene expression shows early response to infection, and it subsequently returns to the basal level to reduce cellular metabolism and store energy for other processes.

Conclusion

This study identified resistant and susceptible Ghanaian rice cultivars to *Xoo* K1 strain infection. Popa exhibited the highest resistance level to *Xoo* among all Ghanaian genotypes. These findings suggest that Popa is a promising candidate cultivar that may be widely utilized in the Ghanaian agricultural system and may contribute to improving BLB disease management. Furthermore, Popa could also be included in plant breeding programs in Ghana using available modern breeding technologies.

In addition, *Xa4* may account for some resistance in Ghanaian rice cultivars to *Xoo*, but marker and phenotypic data were largely inconsistent. While the markers screened for in this study may not provide much insight into resistance status of West African rice cultivars, it highlights the diversity of *R* genes responsible for resistance, and suggests that resistance in West African rice may rely on *Xa*-*R* genes or alleles as yet unreported. This unique West African germplasm will likely be useful in future works determining different modes of action/ mechanisms of resistance, novel resistance alleles or loci, and/or different epistatic interactions related to vast differences in genotypic background.

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Author Contributions

EF, NCH and HHK: conducted the experiments; EF: wrote the manuscript; NKR and WNJ: helped in the experiments; NKR, QMI, BGM, and AH: analyzed the data and reviewed the manuscript for its technical content' BWY: designed and supervised the study, and mobilized funding.

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