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Expression profiles of *XIK1* and *OsSWEET14* genes in parental and backcrossing rice lines after *Xanthomonas oryzae* pv. *oryzae* infection

Atirada Boondech[1], Kawee Sujipuli[1,2], Kumrop Ratanasut[1,2], Tepsuda Rungrat[1,2], Thanita Boonsangsrom[1,2], Niran Aeksiri[1,2], Wittaya Tawong[1,2], Pongsanat Pongcharoen[1,2,*]

[1]Department of Agricultural Science, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok 65000 Thailand
[2]Center of Excellence in Research for Agricultural Biotechnology, Naresuan University, Phitsanulok 65000 Thailand
*Corresponding author: pongsanatp@nu.ac.th

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ABSTRACT *Oryza sativa* L. ssp. *indica* (RD47 cultivar) is a major commercial rice variety known for its highly stable yields. However, it is highly susceptible to bacterial blight disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). While previous research has focused on improving rice cultivars through breeding programs, no reports involved the interaction between *Xoo* infection and gene expression. This study aimed to analyze the relationship between bacterial blight disease and gene expression, focusing on two resistance genes (*Xa21* and *XIK1*) and one susceptible gene (*OsSWEET14*). Gene expression analysis revealed that the *Xa21* gene conferred effective resistance against bacterial blight *Xoo*16PK002 infection, providing high and moderate resistance to bacterial blight symptoms in two rice varieties carrying the *Xa21* gene, IRBB21 and the near–isogenic RD47–*Xa21* BC_4F_4, respectively. Additionally, the *Xa21* gene directly induced *XIK1* expression in both resistance rice cultivars. Moreover, one susceptible gene, *OsSWEET14*, was consistently up–regulated in only the bacterial blight–susceptible indica rice cultivar RD47. Therefore, the up–regulation of resistance genes and the suppression of susceptible genes contributed to the improvement of bacterial blight disease in the RD47 cultivar. *Xa21* emerged as a critically important gene in directly inducing mechanisms against *Xoo*, thereby promoting the reduction of bacterial blight disease.

KEYWORDS Bacterial blight; Gene expression; *Oryza sativa*; Resistance gene; Susceptible gene; *Xanthomonas oryzae* pv. *oryzae*

1. Introduction

Rice (*Oryza sativa*) is a primary global food source. Stable production of rice is paramount to managing food security, notably in developing countries in Asia (Bandumula 2018). The rice bacterial blight (BB) pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causes a yield–reducing disease (Niño-Liu et al. 2006) that impacts rice yield by up to 50% (Liu et al. 2014). Currently, rice BB disease is spreading widely in almost all global rice–growing countries (Jiang et al. 2020). At the molecular level, 22 dominant and 9 recessive BB resistance genes have been identified. The resistance gene *Xa21* (*Os11g0559200*) in plants encodes a receptor–like protein kinase (RLK) and confers resistance against diverse strains of *Xoo* (Aryanti et al. 2016; Nadhira et al. 2022; Song et al. 1995) as a triggered *Xa21*–mediated immunity (Liu et al. 2019). The *Xa21* gene product was accumulated in a plasma membrane and recognized by the tyrosine–sulfated protein RaxX, generated by *Xoo* (Liu et al. 2019). Previous publications reported that the *Xa21* gene mediated BB resistance by activating resistance genes in rice. The *Xoo–induced kinase 1* (*XIK1*) gene acts as a co–receptor of *Xa21*, by positive regulation of *Xa21* that mediates immunity, and *Xa21* gene expression increased under *Xoo*–infected rice (Hu et al. 2015). Nevertheless, the mechanisms by which the *Xa21*–gene mediates BB resistance have not yet been identified.

By contrast, the *OsSWEET* family encoding a sugar transporter is a major BB susceptibility gene targeted by a transcription activator–like (TAL) effector that is secreted by *Xoo*. Among *OsSWEET* genes, *OsSWEET11*, *13* and *14* are the major susceptible targets of bacterial *Xoo* isolated from the field (Antony et al. 2010; Yang et al. 2006; Zhou et al. 2015). In agreement with previous observations, Zeng et al. (2020) found that *OsSWEET11* and *OsSWEET13* were specifically targeted by TAL effectors of *Xoo* strains PthXo1 and PthXo2, respectively, while *OsSWEET14* was commonly targeted by many different TAL effectors from *Xoo* strains of AvrXa7, PthXo3, TalC or Tal5 found in various African and Asian *Xoo* strains (Zeng et al. 2020). These findings suggested that the *OsSWEET14* gene could be a broad–spectrum BB disease, caused by both African and Asian *Xoo* strains.

Molecular methods are now used to investigate the mechanisms between rice genes (or their products) and various pathogen effectors (Oliva et al. 2019; Xu et al. 2022). A previous study showed that gene expression of *XIK1* was earlier induced in the rice cultivar BB–resistant BC_3F_3 (*Xa21*/*Xa21*) than in susceptible cultivar RD47 after *Xoo* pathogen infection. It is possible that *XIK1* was activated earlier in resistant plants than in susceptible cultivars (Sagun et al. 2019). However, the precise mechanism of the *Xoo*–*Xa21* gene interaction remains unclear. To evaluate the relative gene expression profile of resistance and susceptible gene to BB disease, this study characterized the *Xoo*–rice interaction as *Xa21*–mediated resistance. The bacteria isolated of *Xoo*16PK002 was used to infect three different rice cultivars of one–near–isogenic rice genotype carrying the *Xa21* gene, including two genotype controls of IRBB21–donor parent (with the *Xa21* gene) and the RD47–recipient parent (without the *Xa21* gene). The expression profiles of BB resistance gene (*Xa21* and *XIK1*) and susceptible gene (*OsSWEET14*) was characterized in various specific times after *Xoo* inoculation.

2. Materials and Methods

2.1. Plant material

The rice cultivars used in this research included the recipient parent (RD47) and donor parent (IRBB21), carrying the *Xa21* gene resistant to bacterial leaf blight. The marker–assisted backcross breeding (MABB) approach followed a recurrent backcross procedure including four generations of backcross and three generations of self–pollinated (BC_3F_3) called RD47–*Xa21* BC_4F_3–line12 (developed by Sagun et al. (2019). This line was further one backcrossed and one self–pollinated to generate BC_4F_4–line12.

Based on polymerase chain reaction (PCR) assay, two MABB approaches were used to detect three different *Xa21* genotypes (*Xa21*/*Xa21*, *Xa21*/*xa21* and *xa21*/*xa21*) as the pTA248 co–dominant marker pairs that amplified the PCR product, corresponding to approximately 925 and 730 bp for the presence of *Xa21* and *xa21* genes respectively. The *Xa21A* gene specific marker pair (designed from accession no. U72723) was used as the dominant marker for confirming the presence of only *Xa21*, amplifying the PCR product expected to 1152 bp.

The F_1 seed production was cross pollinated between the recipient–RD47 and the donor–IRBB21 parents. The F_1 plants were backcrossed with the recipient parents to raise BC_4F_1 generations. Marker selected plants, heterozygous for the *Xa21* locus in the BC_4F_1 generation were self–pollinated to generate BC_4F_2 generations. In the BC_4F_2 generation, plants homozygous for *Xa21*/*Xa21* were selected with maximum genome recovery of RD47 through marker–assisted background selection followed by stringent phenotyping. The selected plants were then advanced through pedigree based phenotypic selection to obtain *Xa21*/*Xa21* near–isogenic lines.

All BB resistant performance evaluation and molecular marker analyses were conducted in a greenhouse at the Faculty of Agriculture Natural and Resources, Naresuan University, Thailand.

2.2. Bacterial culture

The *Xoo* bacterial culture was isolated from apparently symptomatic BB disease on rice leaves collected from paddy fields in Phitsanulok Province, Thailand. Its isolate, called *Xoo*16PK002, was identified using the assay developed by Buddhachat et al. (2022). The *Xoo*16PK002 was cultured on nutrient agar (0.5% peptone, 0.3% yeast extracts, 0.5% sodium chloride and 1.5% agar). A single bacterial colony was streaked on nutrient agar and then incubated at 28 °C for 24 to 48 h. The *Xoo*16PK002 inoculum was prepared by suspending the bacterial colonies in sterilized distilled water. Bacterial dilution was adjusted to OD_{600} equal to 0.2 before adding a drop of the surfactant Tween–20. Surgical grade scissors were dipped in the *Xoo*16PK002 inoculum and used to cut one centimeter sections of an expanded leaf of a 55 days old plant (Kauffman et al. 1973). The bacterial *Xoo*16PK002 was inoculated to five leaves of each plant of the IRBB21, RD47–*Xa21* BC_4F_4 and RD47 cultivars. For each cultivar, five leaf samples were collected from the area below the clipping lesion, approximately 2 centimeters in size, at specific time points of 0, 1, 2 and 3 hours post inoculation (hpi) with *Xoo*16PK002. Sterilized distilled water was applied for mock inoculation as a control experiment. To determine gene expression profiles, leaf samples were collected at specific times of 0, 1, 2 and 3 hpi. Samples were immediately stored in liquid nitrogen at –80 °C until used. At 21 days after inoculation, lesion length was measured and scored for BB resistance according to the International Rice Research Institute (IRRI) Standard Evaluation System.

2.3. Quantification of gene expression analysis

Leaf samples (100 mg) were extracted for total RNA using Total RNA Extraction Kit Maxi (RBC Real Genomics, Taiwan), according to the manufacturer's instructions. To remove genomic DNA contamination, the total RNA sample was further treated with RNase–Free DNaseI (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, total RNA was assessed for quality and integrity by gel electrophoresis, with concentration quantified by absorbance measurements at A_{260} nm and A_{280} nm using a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Purity was assessed by the ratio of A_{260} nm to A_{280} nm from 1.8 to 2.0.

To synthesize first strand cDNAs, the total RNA template (500 ng) was reverse transcribed in 20 µL reaction mixture using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Baltics, UAB, LT) according to the manufacturer's protocol. The reaction contained 1 µL Oligo (dT)18 primer, 4 µL 5X Reaction buffer, 1 µL RiboLock RNase Inhibitor (20 U/µL), 2 µL 10 mM dNTP

TABLE 1 Primers used for qRT-PCR analysis.

Target gene	Primer name	Sequence (5′–3′)	PCR product	Reference
Xa21	Xa21–F	CAGAGTATGGCGTTGGGCT	114 bp	(Promma et al. 2016)
	Xa21–R	CGGGTCTGAATGTACTGTCA		
XIK1	XIK1–F	TTGGGCCATTGCCAACAAGC	187 bp	(Hu et al. 2015)
	XIK1–R	GTGGTAAGTTTGGCGCACTC		
OsSWEET14	SWEET14–F	GGCGACCGCCGCATCGTGGTT	195 bp	(Verdier et al. 2012)
	SWEET14–R	GCCCAGCACGTTGGGAAGAGCG		
edf	Edf–F	TCCGAACCAGCAGATCATCG	158 bp	(Wang et al. 2016)
	Edf–R	GCATGGTATCAAAAGACCCAGC		

Mix and 1 µL RevertAid M–MuLV RT (200 U/µL), with final volume adjusted to 20 µL using nuclease–free water. The reaction mixture was incubated as three steps of 25 °C for 5 min, 42 °C for 60 min and 70 °C for 5 min. The first strand cDNA was immediately subjected to quantitative real–time PCR (qRT–PCR) amplification and stored at –20 °C until required for further use.

The qRT–PCR reaction (12.5 µL) was conducted in Thermo Scientific Maxima SYBR Green qPCR Master Mix (2X) (no ROX, Thermo Fisher Scientific, Baltics, UAB, LT) (6.5 µL), with 10 µM of each with gene specific primers (Table 1). The qRT–PCR amplification with no template controls (NTCs) were carried out using the Eco48 Real–time PCR system (Eco™48, PCRmax Limited, UK) under the following conditions: 1 cycle of 50 °C for 2 min and 1 cycle of 95 °C for 10 min, followed by 35 cycles (95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s). Finally, a melting curve was realized by progressively heating the reaction mixture from 55 °C to 95 °C using 0.3 °C increments every 0.75 s to check the purity of the qRT–PCR product. The average threshold cycle (Ct) was used to calculate the fold change of gene expression. The expression of interested genes was normalized to the expression of the *Endothelial Differentiation Related Factor* (*edf*) gene (Sagun et al. 2020). The value of $2^{-\Delta\Delta Ct}$ was performed for using the relative quantification method. All reactions were repeated five times with three biological replicates.

2.4. Statistical analysis

All experiments followed a completely randomized design (CRD). Test samples included five plants per rice cultivar (IRBB21, RD47–*Xa21* BC_4F_4 and RD47) with five leaves for each cultivar. One–way analysis of variance and Duncan's Multiple Range Test were used to determine significant ($P < 0.05$) differences in disease development between the IRBB21, RD47–*Xa21* BC_4F_4 and RD47 cultivars using the R program (version 3.2.4; https://www.r–project.org/).

3. Results and Discussion

3.1. Analysis of resistance gene (Xa21 and XIK1) expression after Xoo infection

To establish a platform for studying *Xa21* gene expression, a nearly isogenic line of rice indica variety RD47 carrying the desirable *Xa21* gene was generated by the backcross method. Three 55 days old rice cultivars including IRBB21, RD47–*Xa21* BC_4F_4 and RD47, were investigated for *Xoo*16PK002 infection by the leaf–clipping method. After inoculation, all samples were collected at specific times for qRT–PCR analysis. Results showed that the expression of the *Xa21* gene, conferring a broad resistance against BB disease, was initially up–regulated after *Xoo*16PK002 inoculation for both cultivars IRBB21 (Figure 1a) and RD47–*Xa21* BC_4F_4 (Figure 1b) but not in RD47 (Figure 1c). The expression level of *Xa21* gene in IRBB21 initially increased (2.5 folds) at 1 hpi with significant difference from non–infection. The expression levels continuously increased (3.5 folds) at 2 to 3 hpi, compared to non–infection ($P < 0.05$). Similarly, with the RD47–*Xa21* BC_4F_4 cultivar, the expression of the *Xa21* gene suddenly increased (3.5 folds) at 1 hpi, then decreased after 2 and 3 hpi ($P < 0.05$). By contrast, *Xa21* gene expression was not observed in the RD47 rice cultivar because of *Xa21* absence. These qRT–PCR results indicated that the rice *Xa21* locus was rapidly induced soon after *Xoo*16PK002 infection (1–2 hpi).

The expression profile of the *XIK1* gene was initially induced at 1 hpi and greatly induced at 2 hpi in the IRBB21 cultivar (1.5 folds). The expression level of *XIK1* significantly increased (3.2 folds) at 3 h after *Xoo*16PK002 infection ($P < 0.05$) (Figure 1d). The *XIK1* expression profiles in genes RD47–*Xa21* BC_4F_4 and RD47 were also observed, with similar results to IRBB21. The expression level of *XIK1* in RD47–*Xa21* BC_4F_4 was slightly induced (1.4 folds) after 2 to 3 hpi ($P < 0.05$) (Figure 1e), while the signal of the *XIK1* gene was slightly enhanced after 1 hpi in the RD47 cultivar before continuously increasing (2.5 folds) at 2 hpi and then dropping at 3 hpi (Figure 1f). It was noticed that the *XIK1* gene expression in the IRBB21 cultivar continuously increased (3.2 folds) from 2–3 hpi after bacterial infection, compared to non–infection. However, the highest expression of *XIK1*

gene in RD47–*Xa21* BC4F4 (1.39 fold) and RD47 cultivar (2.45 fold) was observed at 2 hpi. The qRT–PCR results suggested that *Xoo*16PK002 infection of rice leaves led to increased expression of the defense–related genes (*Xa21* and *XIK1*) in both IRBB21 and introgressed RD47–*Xa21* cultivars.

3.2. Expression of susceptibility gene, OsSWEET14, after Xoo infection

The *OsSWEET14*, encoding a sugar transporter, is known as a major susceptibility gene for BB disease targeted by TAL effectors from *Xoo*16PK002 strains. The expression level of the *OsSWEET14* gene gave higher increase in RD47 followed by RD47–*Xa21* BC_4F_4 than in IRBB21 at every time points after *Xoo*16PK002 infection (Figure 1g–i). The *OsSWEET14* gene level was consistently expressed during 0–2 hpi in all tested rice cultivars, compared to the non *Xoo*–infected control. It was noticed that the *OsSWEET14* gene expression in RD47–*Xa21* BC_4F_4 was slightly decreased during 0–2 hpi, then quickly upregulated (2.3 folds) at 3 hpi. On the other hand, *OsSWEET14* gene expression in RD47 cultivar tended to increase during 0–2 hpi, and significantly up–regulated (3.5 folds) at 3 hpi. At 3 hpi, results showed that *Xoo*16PK002 induced the highest *OsSWEET14* gene expression in both RD47–*Xa21* BC_4F_4 (2.3 folds) and RD47 (3.5 folds), compared to IRBB21 at the same time point ($P < 0.05$). By contrast, its gene expression level in IRBB21 was not significantly induced after initial *Xoo*16PK002 infection at all time points. These results indicated that *Xoo*–infection induced up–regulation of *OsSWEET14* gene expression in rice with genetic background from RD47 but not from IRBB21.

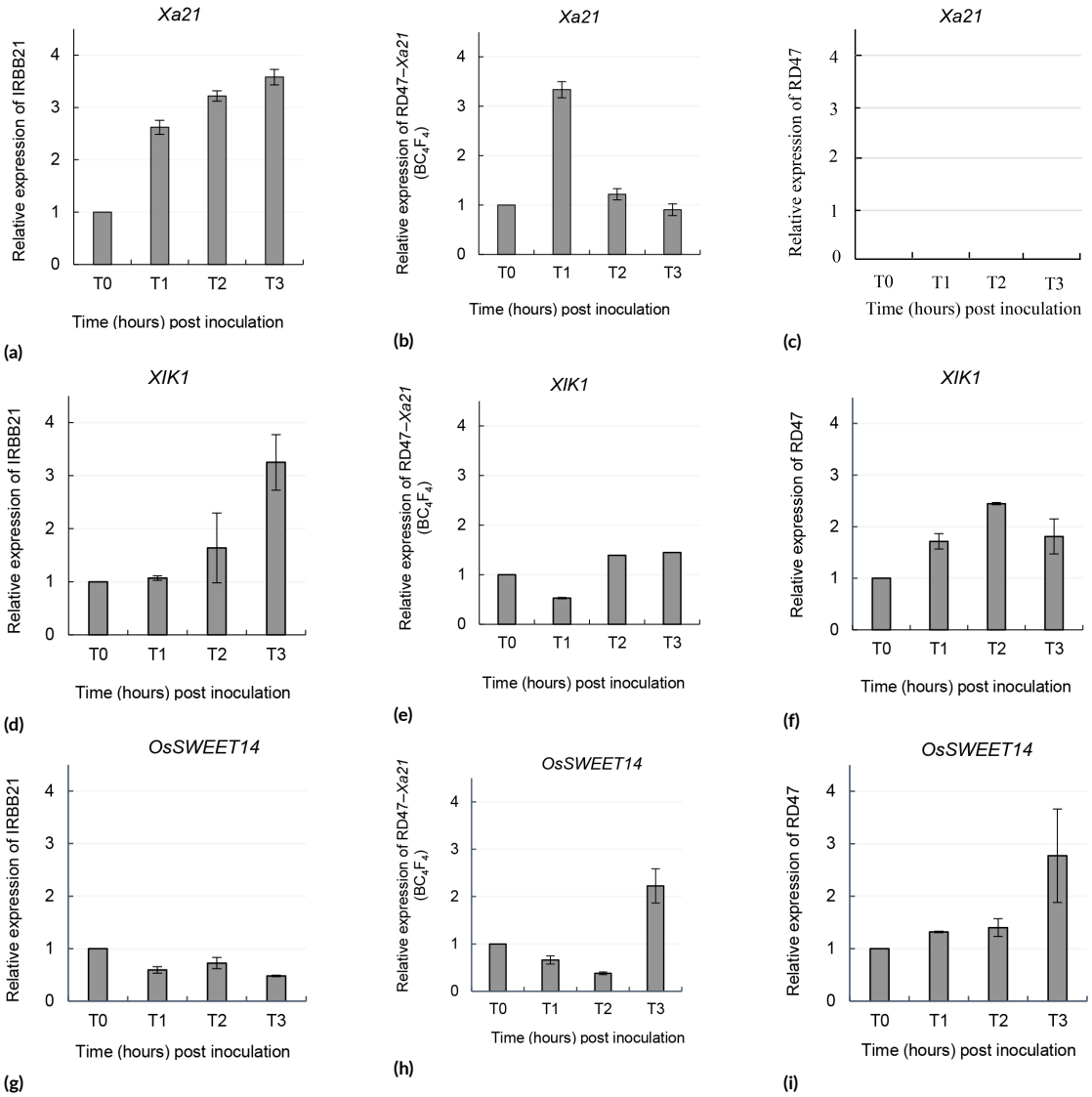

FIGURE 1 Relative expression of resistance genes in (a to c) *Xa21*, (d to f) *XIK1* and susceptibility genes (g to i) *OsSWEET14* in three different rice cultivars after *Xoo*16PK002 inoculation, where 0, 1, 2 and 3 hpi are presented as T0, T1, T2 and T3 with three independent biological replicates. Error bars indicate standard deviation of the analyzed data normalized relative to the *edf* gene.

3.3. Symptomatic BB among rice cultivars through Xoo infection

Stable expression of the *OsSWEET14* indicates more susceptibility to *Xoo*, resistance for *Xa21* and *XIK1* according to various studies. From previous results, the up–regulated expression of *Xa21* and *XIK1* genes and stable expression of the *OsSWEET14* gene in *Xoo*16PK002–treatments (compared to untreated *Xoo*16PK002) in both IRBB21 and RD47–*Xa21* cultivars were identified as *Xoo* resistant. To confirm these *Xoo*–resistant cultivars, 55 days old rice plants were inoculated with *Xoo*16PK002 by the leaf–clipping method, with photographs taken at 14 and 21 days post inoculation under greenhouse conditions (Figure 2). Results showed that both IRBB21 and RD47–*Xa21* cultivars were resistant to *Xoo*16PK002 at 14 days post inoculation, and externally exhibited reduced average lesion length on the leaves at 3.20 ± 0.26 and 12.26 ± 1.95 centimeters, respectively, compared to the RD47 cultivar with higher average lesion length of 36.93 ± 1.92 centimeters ($P < 0.05$). At 21 days after *Xoo*16PK002 inoculation, the IRBB21 cultivar and RD47–*Xa21* carrying *Xa21* showed slow progress of lesion length at 5.50 ± 0.25 and 22.40 ± 1.02 centimeters, respectively, while the RD47 cultivar carrying null–*Xa21* showed highest lesion length at 64.76 ± 1.40 centimeters ($P < 0.05$) (Table 2). Based on the disease severity at 14 days post inoculation, rice cultivars RD47, IRBB21 and RD47–*Xa21* BC_4F_4 were classified into three groups as high susceptibility (lesion length > 20 centimeters), high resistance (lesion length < 5 centimeters) and moderate susceptibility (lesion length 10–15 centimeters). These findings suggested that the RD47–*Xa21* BC_4F_4 line with introgressed *Xa21* was associated with *Xoo*16PK002 resistance increase and BB lesion length reduction.

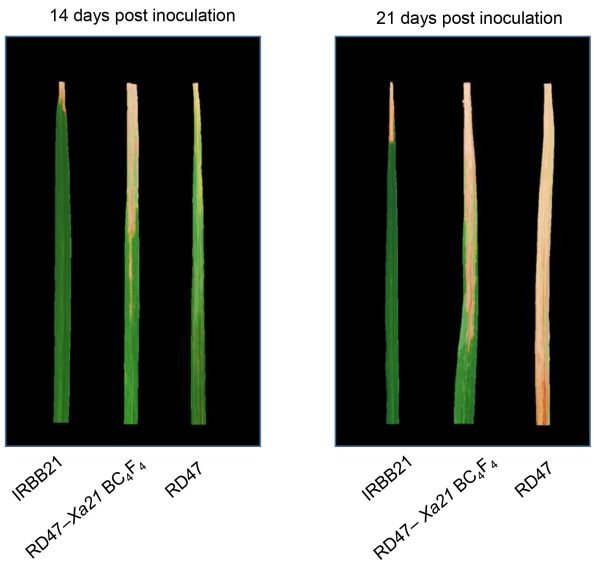

FIGURE 2 Comparison of in–field phenotypes of three rice cultivars after inoculation of *Xoo*16PK002. Disease development of *Xoo*16PK002 isolates on IRBB21, RD47–*Xa21* BC_4F_4 and RD47 after 14 and 21 days post infection. IRBB21 exhibited resistance, RD47–*Xa21* BC_4F_4 exhibited moderate susceptibility, while RD47 exhibited complete susceptibility.

3.4. Discussion

The *Xa21* gene, encoding the predicted innate immune receptor of a receptor–like kinase (RLK), play a specific role in cell–surface recognition of a pathogen ligand. The subsequent activation of an intracellular defense response led to broad resistance protection against BB disease (Thomas et al. 2018; Zhu et al. 2022). The IRBB21 cultivar has been reported resistant to many *Xoo* strains from the Philippines and India (Swamy et al. 2006). The Thai rice cultivar RD47 is widely grown in lower Northern Thailand but is susceptible to BB disease. In this study, the near–isogenic RD47–*Xa21* (BC_4F_4) line 12 was improved by backcrossing and marker–assisted selection of the IRBB21 and RD47 cultivars. This RD47–*Xa21* line was determined for *Xa21*–mediated resistance against *Xoo*16PK002 and BB disease symptoms post bacterial infection. Resistance gene expressions of *Xa21* and *XIK1* were significantly up–regulated at 1 and 2 hpi, respectively in both IRBB21 and RD47–*Xa21*–line12 cultivars compared to RD47 as the recipient parent. Our results agreed with previous findings that the *Xa21* gene is critically important in initiating the signaling pathway associated with enhancing BB resistance against bacterial *Xoo* infection in rice (Thomas et al. 2018). Interaction between the *Xa21* extracellular domain from plants and *Xoo* protein, called Raxx, are key mechanisms that control the robust immune response of *Xa21* (Pruitt et al. 2015). Some defense–related genes in rice were also reported to be involved in interaction with the *Xoo* bacterial strain as co–receptors

TABLE 2 The lesion length and disease severity of three rice cultivars (RD47, IRBB21 and RD47–*Xa21* BC_4F_4) with bacterial isolate of *Xoo*16PK002 under greenhouse condition.

Rice cultivar	Lesion length (centimeters) after inoculation		Disease severity*	
	14 days	21 days	14 days	21 days
RD47	36.93 ± 1.92[a]	64.76 ± 1.40[a]	HS	HS
IRBB21	3.20 ± 0.26[c]	5.50 ± 0.25[c]	R	MR
RD47–*Xa21* BC_4F_4	12.26 ± 1.95[b]	22.40 ± 1.02[b]	MS	HS

*Disease severity: R = resistant, < 5 centimeters; MR = moderate resistant, > 5–10 centimeters; MS = moderate susceptible, > 10–15 centimeters; S = susceptible, > 15–20 centimeters; HS = highly susceptible, > 20 centimeters (Source: IRRI (1996))
Data are presented as the mean±standard deviation of lesion lengths. Different superscripts with in the same column are significantly different ($P < 0.05$) based on Duncan's Multiple Range Test.

of *Xa21* and regulation of *Xa21*–mediated BB resistance (Jiang et al. 2020). Hu et al. (2015) reported that *XIK1* shared a structural motif with *Xa21* that related to *Xa21* defense immunity. Our results indicate that expression levels of *XIK1* were induced at late–stage post inoculation by *Xoo*16PK002 in both the BB resistant IRBB21 and BB susceptible RD47 cultivars. These results suggested that *XIK1* was activated and progressively expressed after *Xoo*16PK002 infection. Concurring with previous findings, the expression of *XIK1* was induced rapidly after infection with the pathogen of *Xoo* (Hu et al. 2015; Sagun et al. 2019). Therefore, the *XIK1* gene might play an important role in co–regulation with *Xa21*–mediated resistance again BB disease in rice.

By contrast, the *OsSWEET14* gene in susceptible rice RD47 background was highly and consistently expressed during *Xoo*16PK002 infection, compared to IRBB21 and RD47–*Xa21* line12 because the *OsSWEET14* gene in RD47 acts as a major susceptibility gene for BB disease caused by *Xoo* strains (Streubel et al. 2013). Susceptible gene (*OsSWEET14*) in IRBB21, was not changed through expression. These finding indicated that *Xoo*–infected rice cultivar IRBB21 trended to induce upregulation of *Xa21* gene and suppress *OsSWEET14* gene, which might subsequently induce expression of other resistant genes, resulting to initiate mechanism of BB resistance in rice. After *Xoo* infection, the TALEs were translated and subsequently guided to interact with target effector–binding elements at the promoter region of the *OsSWEET14* gene, encoding a sugar transporter as the carbon source for bacterial growth. Previous studies have shown that disruption of *OsSWEET14* in Zhonghua 11 promoted strong resistance against the *Xoo* strain (Zeng et al. 2020). The *OsSWEET14* gene was considered a major virulence determinant for *Xoo* isolates (Yang and White 2004; Luo et al. 2021). However, the *OsSWEET14* gene in different rice genetic backgrounds might impact diverse responses of *Xoo* strains. Therefore, broad resistance to BB by the function of the *OsSWEET14* gene in rice requires further experimental confirmation.

The rice IRBB21 and RD47–*Xa21* line12 exhibited high and moderate resistance to BB symptom appearance 21 days after *Xoo*16PK002 inoculation. Moreover, *Xoo*16PK002–infected RD47–*Xa21* plants were not negatively impacted for main agronomics such as size, shape and color of seed under greenhouse condition (data not shown). These results suggested that the resistance rice cultivars (IRBB21 and RD47–*Xa21*) infected by *Xoo* showed increased expression of *Xa21* and *XIK1* genes but decreased expression of the *OsSWEET14* gene. All the studied genes directly induced mechanisms against *Xoo* response with BB disease reduction in the studied rice cultivars.

4. Conclusions

The *Xa21* gene in rice showed effective resistance against *Xoo*16PK002, providing high and moderate resistance to BB symptoms in IRBB21 and backcrossing RD47–*Xa21*, respectively. This *Xa21* and *XIK1* genes rapidly expressed in both IRBB21 and RD47–*Xa21*. The *OsSWEET14* gene was consistently up–regulated in only the RD47 cultivar, causing susceptibility to *Xoo*16PK002. Similarly, both IRBB21 and RD47–*Xa21* cultivars externally exhibited BB–lesion length reduction on the leaves, compared to the RD47 cultivar. This introgressed–*Xa21* approach with marker–assisted selection efficiently improved BB resistance of elite rice cultivars such as RD47 with no detectable yield penalties. Thus, other *Xa21*–mediated resistance genes (such as *OsWRKY* family) and susceptible gene *OsSWEET* family (such as *OsSWEET11*, *OsSWEET13* and *OsSWEET14*) should be further studied to understand valuable insights into BB–resistance mechanism against *Xoo* invasion in rice.

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Authors' contributions

PP conceived the project. PP, KS, KR designed the experiments. KR provided bacterial strain. AB, PP performed the experiments. TR, TB provided all rice cultivars. PP, WT, NA analyzed the data. PP, KS, AB wrote the manuscript. All authors critically reviewed the manuscript and approved the final version to be published.

Competing interests

The authors declare no conflict of interest.

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