ORIGINAL RESEARCH

Salicylic acid application against bacterial blight resistance in *Xa21*-introgression Thai rice cultivar 'Phitsanulok 2'

Natchanon Meesa[1], Kawee Sujipuli [2,3], Kumrop Ratanasut [2,3], Pongsanat Pongcharoen [2,3], Tepsuda Rungrat [2], Thanita Boonsrangsom [2,3], Wanwarang Pathaichindachote [2,3], Phithak Inthima [1,3]*

[1]Department of Biology, Faculty of Science, Naresuan University, 99 Moo 9, Tha Pho, Mueang, Phitsanulok 65000, Thailand

[2]Department of Agricultural Science, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, 99 Moo 9, Tha Pho, Mueang, Phitsanulok 65000, Thailand

[3]Center of Excellence in Research for Agricultural Biotechnology, Naresuan University, 99 Moo 9, Tha Pho, Mueang, Phitsanulok 65000, Thailand

* To whom correspondence should be addressed. Email: phithaki@nu.ac.th

Abstract

Rice cultivar 'Phitsanulok 2' ('PSL2') is popularly grown in lower-northern Thailand, because it has good agricultural traits with high-quality and high-yield grains. However, one significant limitation of rice 'PSL2' is its susceptibility to bacterial blight (BB) disease caused by bacteria *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), leading to considerable yield losses. Effort approaches have been used to enhance the BB resistance of rice 'PSL2' by introducing the resistance gene *Xa21* and using salicylic acid (SA). Thus, the objective of this study was to determine the effects of SA application on BB disease reduction in rice cultivars ('PSL2', 'IRBB21', and backcrossed lines 'PSL2-*Xa21*' in BC_4F_6) after *Xoo*16PK002 inoculation, compared to a mock treatment control. The results showed that the SA-pretreated rice (2 mM) exhibited significantly induced upregulation of the *Xa21* gene expression before the *Xoo*16PK002-infected rice cultivars 'IRBB21' and 'PSL2-*Xa21*-BC_4F_6'. Additionally, the SA-pretreated rice 'PSL2-*Xa21*-BC_4F_6' demonstrated significant suppression of *Xoo*16PK002 proliferation (from 1.4 to 2.4-fold reduction), compared to the H_2O pretreatment. Similarly, the SA-pretreated rice 'PSL2-*Xa21*-BC_4F_6' exhibited increased resistance against BB disease, i.e. reduced lesion lengths (10.0–10.9 cm at 14 DAI and 24.6–28.0 cm at 21 DAI), compared to the water pretreatment (16.8–18.0 cm at 14 DAI and 34.9–35.1 cm at 21 DAI). In summary, the application of the SA-pretreatment in the rice introgression lines 'PSL2-*Xa21*-BC_4F_6' resulted in improved defense mechanisms against bacteria *Xoo*16PK002 through reduced appearance of symptoms and inhibited *Xoo* multiplication.

Keywords

backcross; *Oryza sativa* L.; resistance gene; SAR; *Xanthomonas oryzae* pv. *oryzae*; *Xoo*

1. Introduction

Rice (*Oryza sativa* L.; *Os*) is one of the most important economic crops serving as a primary food source for over half of the global population (Duong et al., 2023; Taratima et al., 2023). Thailand stands out as one of the world's leading rice producers and exporters, with a production of 32.98 million tons in 2021 and 34.32 million tons in 2022 (FAOSTAT, 2021). This notable achievement is further enhanced by the country's diverse array of rice germplasm. The most well-known Thai rice cultivars include fragrant rice (Khao Dawk Mali 105; 'KDML105'), non-fragrant rice (Phitsan-

ulok 2; 'PSL2'), and glutinous rice ('Niaw Ubon'). Among these, the Thai-indica rice cultivar 'PSL2' is extensively grown in Phitsanulok province, Thailand. The rice 'PSL2' was improved through a three-way cross between the 'CNTLR81122-PSL-37-2-1' / 'SPRLR81041-195-2-1' // 'IR56' lines by the Phitsanulok Rice Research Center during 1990–2000. It exhibits favorable agricultural traits, such as high yield productivity, grain quality, and resistance to brown planthopper and green rice leafhopper (Phitsanulok Rice Research Center, Thailand). However, one disadvantage of the 'PSL2' cultivar is its susceptibility to bacterial blight (BB) disease caused by the bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Phitsanulok Rice Research Center, Thailand), leading to significant yield losses ranging from 30% to 60% (Yasmin et al., 2017). Currently, there are no effective methods for preventing the spread of bacterial *Xoo*, but most management strategies involve a combination of cultural, chemical, and genetic approaches (Khoa et al., 2017). One effective approach to decrease the severity of BB disease symptoms is the generation of an introgression line of resistance (*R*) genes to *Xoo* in the rice cultivar 'PSL2'.

Of these *R* genes, the dominant *Xa21* gene encodes a receptor-like protein kinase (RLK) and confers broad-spectrum resistance against Asian *Xoo* strains, triggering *Xa21*-mediated immunity (Peng et al., 2015). The RLK protein recognizes a specific Rax protein secreted by *Xoo* during rice infection (Liu et al., 2019). The formation of the RLK-Rax complex triggers a cascade of signaling events within the plant cell, leading to the activation of *Xa21*-mediated immune response pathways and defense-related proteins, including hypersensitive response (HR) programmed cell death at the site of infection (Radojičić et al., 2018). Thus, to enhance their resistance to BB disease, the *Xa21* gene has been successfully introgressed into many susceptible rice cultivars, such as 'RD47' (Suachaowna et al., 2017), 'CB174R' (Govintharaj et al., 2021), and 'Ciherang' (Biswas et al., 2021) through marker-assisted selection in backcross breeding. For these reasons, introgression lines of 'PSL2-*Xa21*' in the BC_4F_5 generations were produced from a 'PSL2' × 'IRBB21' cross, followed by backcrossing to 'PSL2' for four generations, and then selfing for five generations during 2018–2021. In each generation, two gene-specific markers, pTA248 (tightly linked to the *Xa21* gene) and *Xa21*, were used to identify the homozygous dominant genotype (*Xa21/Xa21*) in 'PSL2-*Xa21*'.

Previous studies have reported that the *Xa21* gene can also trigger a systemic acquired resistance (SAR) response in rice, allowing the rice to increase a long-lasting defense response throughout the entire plant. Additionally, salicylic acid (SA) plays a crucial role in plant defense against various pathogens. For example, SA accumulation triggers a series of responses, such as the activation of defense-related genes (Liang et al., 2022), restricts the growth of pathogenic *Xoo*, and reduces the BB disease severity in the Thai rice cultivar KDML105 (Thanh et al., 2017). The endogenous SA acts as a signal transducer (SA receptor) to subsequently induce the expression of genes related to the defense protein pathway, such as *xa5* (Bimolata et al., 2015), *OsNPR1* and *OsTGA* (Jiang et al., 2020), and *OsPR1* (Schwessinger & Ronald, 2012). Moreover, SA enables the promotion of expression of genes *OsWRKY45* and *OsNAC4* related to the cell death pathway (Duan et al., 2014).

Overall, the combined interactions of the *Xa21* gene and SA application might contribute to enhanced BB resistance, reduce *Xoo* proliferation, and limit *Xoo* spread during bacterial *Xoo* invasion. Thus, the interaction of the SA application and the *Xa21* gene should be studied in response to BB disease symptoms in the introgression rice line 'PSL2-*Xa21*' in the BC_4F_5 generation.

2. Materials and methods

2.1. Rice materials and plant genotyping

The rice introgression lines of 'PSL2-*Xa21*' in the BC_4F_5 generation were produced by marker-assisted selection (MAS) in a backcross breeding approach at the Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Thailand. This process took place in greenhouse conditions with natural ventilation and sunlight, where the temperature ranged from 28.4 °C to 35.2 °C during the day and from 27.6 °C
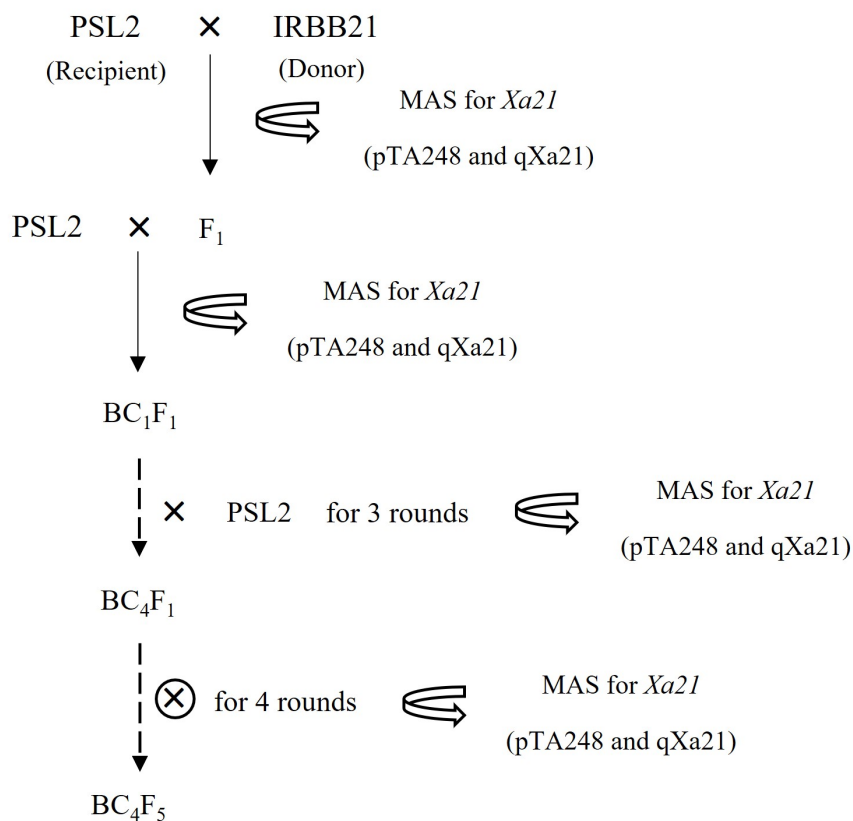
PSL2   ×   IRBB21
(Recipient)   (Donor)

MAS for *Xa21*

(pTA248 and qXa21)

PSL2   × F_1

MAS for *Xa21*

(pTA248 and qXa21)

BC_1F_1

×   PSL2   for 3 rounds

MAS for *Xa21*

(pTA248 and qXa21)

BC_4F_1

⊗   for 4 rounds

MAS for *Xa21*

(pTA248 and qXa21)

BC_4F_5

Figure 1 Schematic flowchart illustrating the introgression of the bacterial blight (BB) resistance gene, *Xa21*, from rice cultivar 'IRBB21' into 'PSL2' through the marker-assisted backcross (MAS) breeding approach. The two MAS markers used are pTA248 and q*Xa21*. In each generation, eight introgression lines carrying the *Xa21* gene are selected for further use.

to 32.9 °C at night, with natural humidity levels between 63.5% and 92.9%. Briefly, the favorable *Xa21* gene from rice cultivar 'IRBB21' (as the donor parent) was introgressed into 'PSL2' (as the recipient parent) to produce F_1 seeds with the crossing method. These F_1 plants were backcrossed to four 'PSL2' generations (BC_4F_1) to increase the genetic background of 'PSL2'. Subsequently, the BC_4F_1 plants were self-pollinated for four generations to generate BC_4F_5 progenies to increase homozygosity (Figure 1). In each generation, the presence of the *Xa21* locus was identified using a direct PCR assay with two specific MAS sets.

The first marker, pTA248, was a co-dominance marker closely linked to the dominant *Xa21* allele, which can amplify two different PCR products corresponding to 730 bp for the null-*Xa21* allele (or recessive *Xa21* allele) and 925 bp for the *Xa21* allele (Yan-chang et al., 2004). The second marker, qXa21, was a dominance marker designed to specifically amplify a 114-bp DNA fragment of the published *Xa21* sequence (Gen-Bank no. U72723).

The base sequences of both markers are listed in Table 1. The selected plants 'PSL2-*Xa21*-BC_4F_5' were naturally grown and evaluated with similar and better agronomic traits than the recipient parent 'PSL2' in field conditions at Phitsanulok Rice Research Center, Thailand.

2.2. PCR amplification

The rice cultivars of parents ('PSL2' and 'IRBB21'), 'PSL2-*Xa21*-BC_4F_1', and 'PSL2-*Xa21*-BC_4F_5' were assigned the genotype of *Xa21/Xa21*, *Xa21/xa21*, and *xa21/xa21* using MAS with a direct PCR approach of Phire Plant Direct PCR Master Mix®

Table 1 Lists of primers and sequences used in this study.

Primer name	Primer sequence (5′-3′)	Amplicon size (bp)	Reference
pTA248-F	5′-AGACGCG GAAGGGTGGTTCCCGGA-3′	925 (*Xa21*), 730 (*xa21*)	Yan-chang et al. (2004)
pTA248-R	5′-*AGACGCGGTAATCGAAAGATGAAA*-3′		
qXa21-F	5′-CAGAGTATGGCGTTGGGCT-3′	114	Promma et al. (2016)
qXa21-R	5′-*CGGGTCTGAATGTACTGTCA*-3′		
Edf-F	5′-TCCGAACCAGCAGATCATCG-3′	158	Sagun et al. (2020)
Edf-R	5′-*GCATGGTATCAAAAGACCCAGC*-3′		
Xoo4009-F3	5′-GTTCACCCTGCCCTTCATTTCCGTCGTCATC-3′	302	Buddhachat et al. (2021)
Xoo4009-B3	5′-*CCTTTGAGATCGCATGCATGAAGAACCACCACA*-3′		

(Thermo Fisher Scientific, UK) according to the manufacturer's protocol. Briefly, leaf samples (1 mm) were crushed in 20 μL of dilution buffer and incubated for 5 min for genomic DNA (gDNA) extraction. The PCR reaction (20 μL) was prepared from the mixture of 1 μL of gDNA template, 1X Phire Direct PCR master mix, and 0.5 μM of each primer. This reaction was performed for DNA amplification in the following PCR conditions: pre-denaturation at 95 °C for 5 min, then 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s, and final extension at 72 °C for 5 min. The amplified PCR products were detected by 1.2% agarose gel electrophoresis.

2.3. Exogenous SA-pretreated and artificially *Xoo*-infected rice assay

Fifty-five-day-old rice plants (specifically during the flourishing tillering stage) were subjected to experimental foliar spray (50 mL/plant) once a day for two consecutive days with different concentrations of SA (0, 1, and 2 mM) on the whole plant. On the next day of pretreatment, five mature top leaves per plant were infected with *Xoo*16PK002 in greenhouse conditions. A distilled water (H_2O)-treated rice leaf was used as the experimental control. In this experiment, the *Xoo*16PK002 bacterium was isolated from paddy fields in Phitsanulok province of Thailand, and was identified using an assay developed by Buddhachat et al. (2021). Meanwhile, the *Xoo*16PK002 culture and inoculation were carried out using the leaf-clipping method (Ke et al., 2017). At 7 and 14 days after inoculation (DAI), lesion lengths of three randomly sampled plants were measured and scored. The scoring criteria were as follows: high resistance (R, lesion length 0–5 cm), moderate resistance (M, lesion length >5–10 cm), moderately susceptible (MS, lesion length >10–15 cm), and susceptible (S, lesion length >15 cm) according to the IRRI Standard Evaluation System (International Rice Research Institute [IRRI], 2013).

The optimal SA concentration was selected for the quantification of gene expression profiles. The leaf samples were collected from the area below the clipping lesion, approximately 2 cm in size, at specific time points of 0, 1, and 2 hours after inoculation (HAI) with *Xoo*16PK002. Upon collection, the samples were immediately immersed in liquid nitrogen and stored at −80 °C until further use for gene expression analysis.

Meanwhile, in the same experimental sample above, leaf samples (1 × 1 cm^2) from the inoculated area below the clipping lesion (approximately 2 cm) of both exogenous SA- and H_2O-pretreated rice plants were harvested at 3 DAI, following a modified method based on Thanh et al. (2017). The harvested sample was followed by surface sterilization with sterilized distilled water and 70% ethanol. Subsequently, the leaf sample was finely chopped into small pieces and placed into sterilized distilled water (100 μL). The mixture was then incubated at 95 °C for 10 min. The supernatant (2 μL) of crude plant extracts was directly used for *Xoo* quantification using the qPCR system with the Xoo4009-F/R marker pair (Table 1). The remaining supernatant was stored at −20 °C for further use.

The symptomatic response to BB disease was evaluated and photographed at 14 and 21 DAI by measuring the lesion lengths of three plants, and then scored according to the IRRI Standard Evaluation System (IRRI, 2013).

2.4. qPCR analysis

The expression profile of the *Xa21* gene in the rice samples was determined using the qPCR assay. Total RNA was extracted from a leaf region below the clipping lesion (approximately 5 cm) using the FavorPrep™ Plant Total RNA Mini Kit (Favorgen, Taiwan) following the manufacturer's instructions. The genomic DNA contamination in the total RNA sample was further eliminated with RNase–Free DNase I treatment (Thermo Scientific, USA). Subsequently, the quality and integrity of the total RNA were evaluated through gel electrophoresis, and the concentration was determined by measuring absorbance at A_{260} nm and A_{280} nm using a NanoDrop™ Lite Spectrophotometer (Thermo Fisher Scientific, USA). The pure RNA sample was indicated by a value of the A_{260}/A_{280} ratio ranging from 1.8 to 2.0. First-strand cDNA synthesis was performed using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Baltics, UAB, LT) according to the manufacture's protocol. The qRT-PCR reaction was carried out using the Thermo Scientific Maxima SYBR Green qPCR Master Mix (Thermo Fisher Scientific, Baltics, UAB, LT) on the Eco48 Real-Time PCR system (Eco™48, PCRmax Limited, UK). The thermal cycling conditions were as follows: an initial denaturation step at 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). 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 (C_t) value was used to calculate the fold change of gene expression. The expression of the *Xa21* gene was normalized to the expression of the *Endothelial Differentiation Related Factor* (*edf*) gene (Sagun et al., 2020). The $2^{-\Delta\Delta Ct}$ method was applied for relative quantification (Livak & Schmittgen, 2001). All reactions were technically repeated three times with at least three biological replicates. The primer sequences used in the qPCR assay are listed in Table 1.

Moreover, the number of bacterial *Xoo* copies (CFU/mL) was quantified by the thermal cycle of qPCR on the Eco48 Real-Time PCR system (Eco™48, PCRmax Limited, UK) in the following conditions: 1 cycle of pre-denaturation at 95 °C for 5 min, followed by 40 cycles (95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s), and final extension at 72 °C for 5 min. The C_t values obtained from the *Xoo* sample were used to calculate the bacterial numbers by comparing them with the standard curve. This curve was generated from a 10-fold serial dilution of the *Xoo* cell suspension (ranging from 25 to 2.5×10^8 CFU/mL) of known concentrations from the initial *Xoo* cell suspension at $OD_{600} = 0.2$ (equivalent to 2.5×10^8 CFU/mL).

2.5. Statistical analysis

The experiment was conducted using a complete randomized design (CRD), with three to five biological replicates. The results were expressed as the mean value ± standard error. Data analysis was performed using IBM SPSS Statistics version 19. Statistically significant differences among the treatment means were determined using Duncan's new multiple range test (DMRT) at *p* value ≤ 0.05 or 0.01.

3. Results

3.1. Marker-assisted selection of the *Xa21* gene

To identify the *Xa21* genotypes across the rice cultivars, the gDNA template extracted from nine introgression lines ('PSL-*Xa21*-BC$_4$F$_5$'), 'PSL-*Xa21*-BC$_4$F$_1$', and two parental lines ('PSL2' and 'IRBB21') was used to amplify the PCR product with two functional pTA248 and qXa21 markers related to BB disease resistance. Based on the pTA248 co-dominant marker analysis, the homozygous dominant genotype (*Xa21*/*Xa21*) was found in the rice cultivar 'IRBB21' and all introgression lines 'PSL-*Xa21*-BC$_4$F$_5$', which amplified a single 925-bp PCR product. The introgression line 'BC$_4$F$_1$' plant exhibited both 730-bp and 925-bp PCR products corresponding to the heterozygous *Xa21* genotype (*Xa21*/*xa21*). In turn, the 'PSL2' showed a single 730-bp PCR product, confirming the homozygous recessive genotype (*xa21*/*xa21*) (Figure 2A).
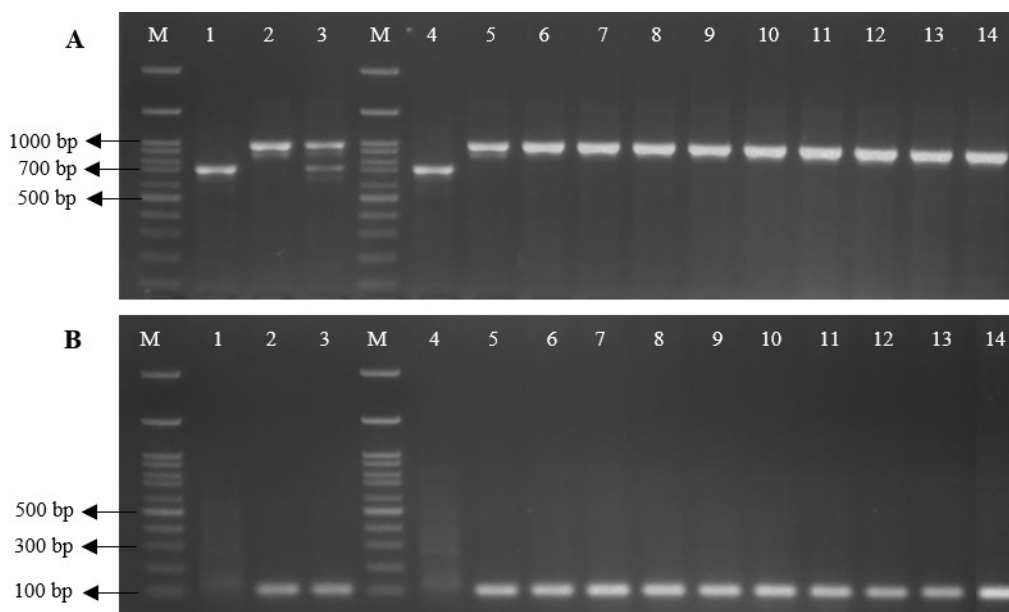
Figure 2 Plant genotyping analysis across 12 rice cultivars ('PSL2', 'IRBB21', 'BC$_4$F$_1$', and 'BC$_4$F$_5$L1-L9' plants) using the pTA248 (**A**) and qXa21 (**B**) markers. In each PCR reaction, the DNA template was derived from the rice cultivar 'PSL2' (lane 1 and 4); 'IRBB21' (lane 2 and 5); 'BC$_4$F$_1$' (lane 3); 'BC$_4$F$_5$L1'–'BC$_4$F$_5$L9' (lane 6–14), listed in Table 2. Lane M represents the 100 bp DNA ladder.

Similarly, the presence of the *Xa21* genotypes among these introgression lines 'PSL-*Xa21*' lines was confirmed using the qXa21-F/R dominant marker. The result showed that all the BC$_4$F$_5$ lines exhibited only a single PCR product, corresponding to a 114-bp long product (Figure 2B), indicating that all the BC$_4$F$_5$ lines contained the homozygous *Xa21* genotype (*Xa21/Xa21*) (Figure 2B). These findings provide strong evidence that all the 'PSL-*Xa21*-BC$_4$F$_5$' lines possess the homologous dominant alleles of the *Xa21* resistance gene.

3.2. Agronomic performance of introgression lines

The selected introgression lines carrying the homozygous *Xa21* genotype underwent a comprehensive evaluation of their agronomic performance in field conditions. The results revealed that the nine introgression lines (without *Xoo*16PK002 infection) exhibited similar or better agronomic performances (days to flowering, plant height, panicle number and length, filled grains per panicle, 100-grain weight, and total grain weight per plant) than the recipient parent 'PSL2' (Table 2). These findings indicate the successful incorporation of the *Xa21* gene without compromising the overall agronomic performance.

Furthermore, all the tested introgression lines also showed a high level of BB resistance, as evidenced by the lesion lengths ranging from 4.80 ± 1.9 cm to 3.8 ± 0.33 cm at 21 days after the *Xoo*16PK002 inoculation, compared to the BB-resistant cultivar 'IRBB21' and the BB-susceptible cultivar 'PSL2' (Table 2, Figure 3). Among these, two introgression plants, BC$_4$F$_5$-L1 and BC$_4$F$_5$-L8, had the highest total grain weight per plant, measuring 89.01 ± 10.66 g and 90.04 ± 3.52 g, respectively. Based on these promising results, these two selected lines were further propagated through self-pollination to generate 'BC$_4$F$_6$' seeds and used for further experiments.

3.3. Effect of exogenous SA pretreatment on BB resistance

To optimize the exogenous SA concentration for BB resistance in rice, whole plants of 'PSL2' (at 55 days old) were pretreated with either SA (0.5, 1, and 2 mM) or H$_2$O, and the following day, the plants were inoculated with the *Xoo*16PK002 isolate in greenhouse conditions. Overall, the results showed that the higher concentrations of SA trended towards reducing the lesion length in BB disease. Among these, the SA pretreatment with 2 mM significantly reduced the severity of BB in

Table 2 Morpho-agronomic performances of nine-introgression lines 'PSL2-$Xa21$-BC$_4$F$_5$' relative to their donor and recipient parents.

Line	Days to flowering	Height (cm)	Panicle/plant	Panicle length (cm)	Filled grain/panicle	100-grain weight (g)	Total grain weight (g)	Lesion length (cm)	Disease severity score*
'BC$_4$F$_5$-L1'	83 ± 0ab	122.5 ± 2.85	19.75 ± 1.94a	31.0825 ± 0.23ab	151.5 ± 5.32ab	3.77 ± 0.09ab	89.01 ± 10.66a	4.8 ± 0.190b	R
'BC$_4$F$_5$-L2'	83 ± 0.41ab	125.25 ± 0.63	17.25 ± 1.19abc	30.7875 ± 0.82ab	126.5 ± 5.37bc	3.81 ± 0.1a	67.4 ± 4.23bcd	3.9 ± 0.22b	R
'BC$_4$F$_5$-L3'	82.25 ± 0.25ab	123.5 ± 1.56	14 ± 0.92c	29.9125 ± 0.54b	156.5 ± 7.86a	3.71 ± 0.15ab	55.29 ± 2.41d	3.9 ± 0.23b	R
'BC$_4$F$_5$-L4'	82.5 ± 0.65ab	127 ± 0.92	15.25 ± 0.86bc	30.12 ± 0.21b	144 ± 13.3ab	3.57 ± 0.11ab	54.11 ± 3.28d	4.525 ± 0.42b	R
'BC$_4$F$_5$-L5'	82.5 ± 0.65ab	124.5 ± 2.4	16.75 ± 1.71abc	30.745 ± 0.32ab	150.75 ± 6.29ab	3.6 ± 0.11ab	67.97 ± 2.06bcd	4.225 ± 0.39b	R
'BC$_4$F$_5$-L6'	83 ± 0.41ab	127.25 ± 1.61	20.5 ± 1.2a	30.4975 ± 0.36b	154 ± 10.74ab	3.485 ± 0.05b	79.91 ± 5.28ab	5 ± 0.39b	R
'BC$_4$F$_5$-L7'	81.75 ± 0.48b	127.75 ± 1.38	14.5 ± 1.33c	30.08 ± 0.42b	155.5 ± 9.08a	3.72 ± 0.05ab	64.72 ± 10.15bcd	4.2 ± 0.31b	R
'BC$_4$F$_5$-L8'	82 ± 0.71b	125 ± 1.09	18.5 ± 1.05ab	30.2025 ± 0.21b	154 ± 4.71ab	3.75 ± 0.06ab	90.04 ± 3.52a	3.8 ± 0.33b	R
'BC$_4$F$_5$-L9'	82 ± 0.41b	127.5 ± 0.65	15.5 ± 0.87bc	31.915 ± 0.38a	151 ± 8.35ab	3.68 ± 0.03ab	64.43 ± 2.72bcd	5.25 ± 0.16b	R
'IRBB21' (donor parent)	82.5 ± 0.87ab	123 ± 2.28	7.5 ± 0.65d	28.215 ± 0.24c	114.75 ± 7.44c	2.52 ± 0.14c	59.94 ± 2.70cd	4.425 ± 0.21b	R
'PSL2' (recipient parent)	81.5 ± 0.29b	123.5 ± 2.07	14.5 ± 1.26c	30.72 ± 0.28ab	143 ± 12.18ab	3.63 ± 0.15ab	74.38 ± 10.2bc	48.47 ± 2.42a	S

All the studied rice cultivars were grown in separate pots in natural greenhouse conditions. Data are expressed as the mean value ± standard error of at least 3 biological replicates. Different letters within the same column indicate statistically significant differences analyzed by DMRT at $p \leq 0.01$. * R, high resistance; S, susceptibility.

'IRBB21'   'PSL2'

'PSL2-*Xa21*-BC$_4$F$_5$-L1'   'PSL2-*Xa21*-BC$_4$F$_5$-L8'

Figure 3 Bacterial blight (BB) lesions on the leaves of the studied rice cultivars. The BB lesions on the leaves of the recipient parent 'PSL2', the donor parent 'IRBB21', and the two selected introgression lines 'PSL2-*Xa21* BC$_4$F$_5$' plants were measured at 21 days after *Xoo*16PK002 inoculation.

the rice 'PSL2' at 7 DAI, with a lesion length on the leaf of 6.35 ± 0.11 cm, compared to the other treatments (Figure 4). Similarly, at 14 DAI, the SA pretreatment with both 1 or 2 mM resulted in a more significant reduction of BB lesion length (15.41 ± 0.24 and 15.10 ± 0.31 cm, respectively) than the H$_2$O pretreatment control (16.47 ± 0.39 cm) (Figure 4). These findings indicated that the exogenous SA pretreatment could enhance systemic BB resistance against *Xoo*16PK002 in the rice cultivar 'PSL2'. Therefore, the role of the SA-mediated gene (such as *Xa21*) conferring BB disease resistance should be further studied.

3.4. SA-mediated *Xa21* expression in the introgression line 'PSL2-*Xa21*'

To determine the influence of the SA pretreatment on the *Xa21*-gene expression in rice responding to *Xoo*, two-introgression lines ('PSL2-*Xa21*-BC$_4$F$_6$-L1 and L8') were experimentally subjected to SA (2 mM) or H$_2$O pretreatment, and then subsequently infected with the *Xoo*16PK002 isolate in greenhouse conditions. Leaf samples were collected at different time points (0, 1, and 2 hours after inoculation; HAI) to quantify gene expression with the qRT-PCR assay. In the SA-pretreated rice cultivars 'IRBB21' and 'PSL2-*Xa21*-BC$_4$F$_6$-L1 and L8', the *Xa21* gene showed slightly higher expression of *Xoo*16PK002 at 0 HAI, compared to the mock pretreatment (Figure 5A). After that, *Xa21* trended to be slightly down-regulated at 1 and 2 HAI of *Xoo*16PK002, compared to 0 HAI of *Xoo*16PK002 under the SA pretreatment (Figure 5A). These results indicated that the SA-pretreated rice might stimulate the *Xa21* gene expression before
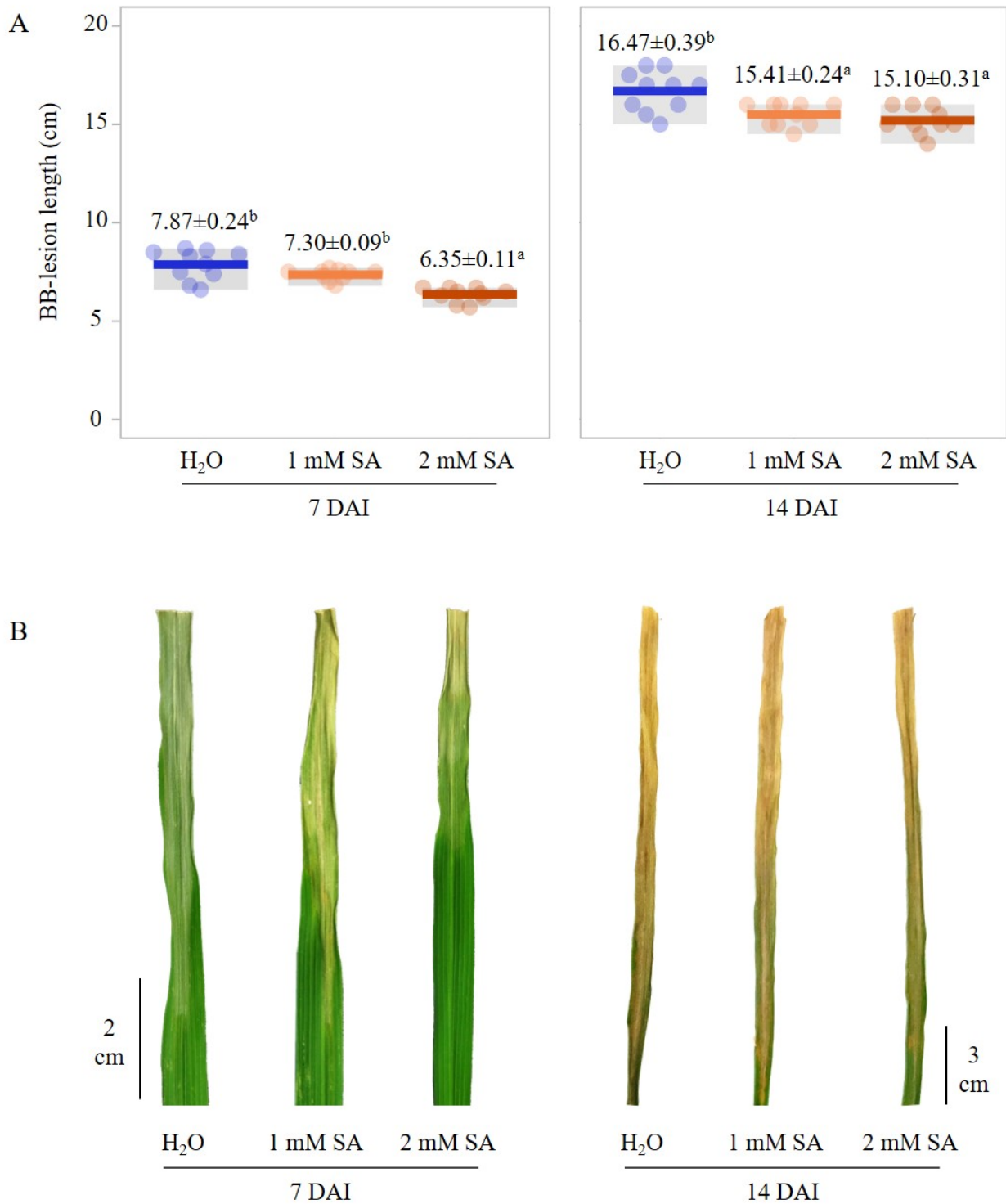
Figure 4 Effect of different concentrations of the SA pretreatment on lesion length (**A**) and symptomatic (**B**) progression of BB disease in rice 'PSL2' at 7 and 14 DAI. Fifty-five-day-old rice plants (at the flourishing tillering stage) were pretreated twice with SA (1 and 2 mM) or H_2O on consecutive days (on days 55 and 56 after plantation) and then subsequently inoculated with the *Xoo*16PK002 isolate on day 57 after plantation.

*Xoo*16PK002 infected the rice. In contrast, under the H_2O pretreatment, *Xa21* showed higher expression at 2 HAI of *Xoo*16PK002 in all the studied rice cultivars, compared to 0 HAI of *Xoo*16PK002 (Figure 5A). However, no *Xa21* gene expression was detected in the rice 'PSL2' carrying a homozygous recessive genotype (*xa21/xa21*) (Figure 5A). Summarized results indicated that *Xa21* gene expression could be induced by either exogenously SA-pretreated or post *Xoo*-infected rice.
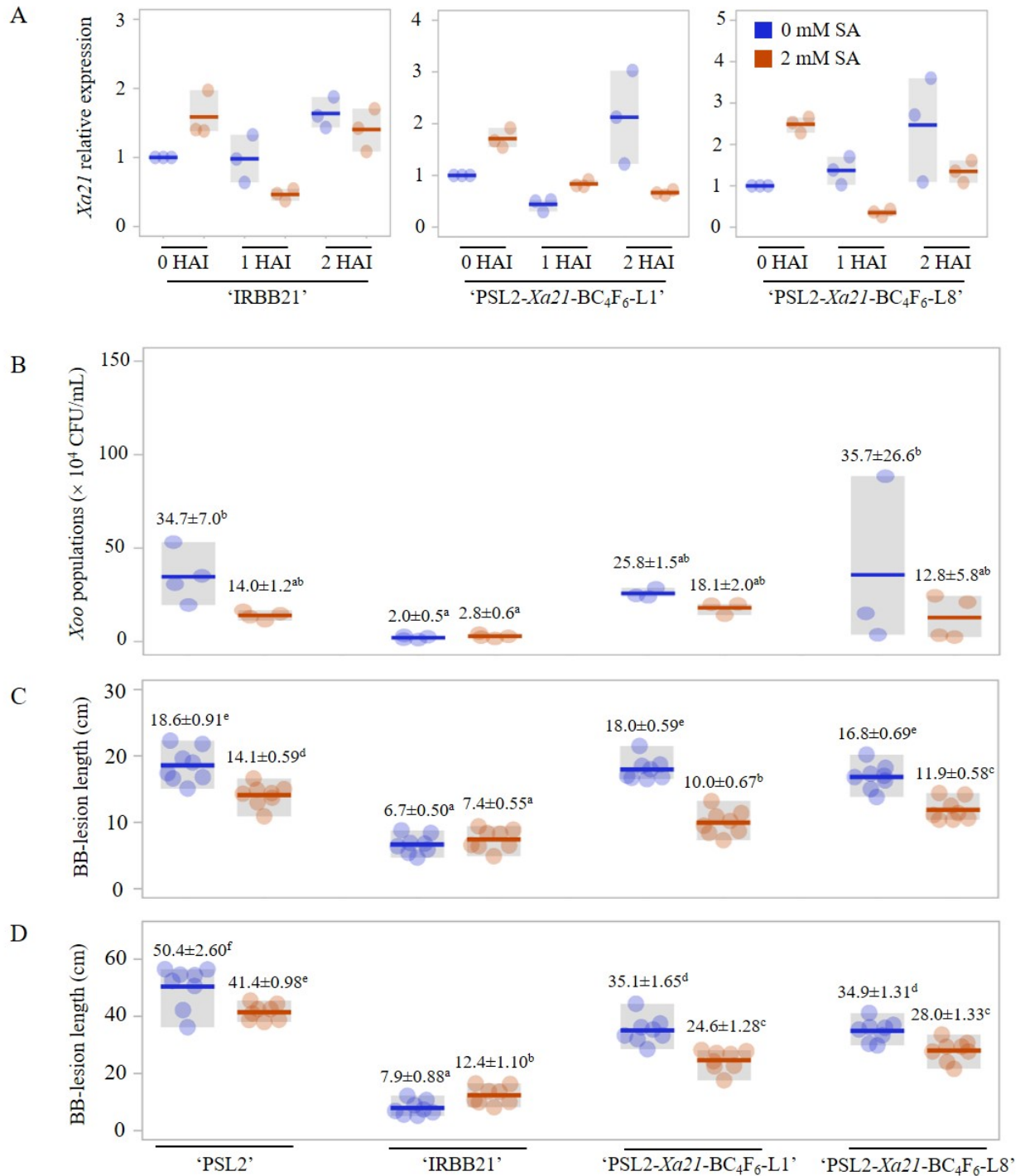
Figure 5 Influence of the SA pretreatment (2 mM) on *Xa21* expression and BB disease resistance in rice introgression lines. (**A**) *Xa21* gene expression in the conditions of SA (2 mM) or H_2O pretreatment and *Xoo*16PK002-inoculated rice cultivars ('IRBB21', 'PSL2', and introgression lines) at 0, 1, and 2 HAI; (**B**) Bacterial populations of *Xoo*16PK002 in rice plants. The number of bacteria in 1 cm leaf sections from the cut sites was measured at 3 DAI; (**C–D**) Lesion length on leaves of rice cultivars ('IRBB21', 'PSL2', and two-introgression lines) infected with the *Xoo*16PK002 isolate at 14 (**C**) and 21 DAI (**D**). Values are the mean ± SE of three independent biological replicates. Letters denote significant differences based on analyses of plants treated with SA and H_2O using the DMRT ($p \leq 0.05$).

To assess *Xoo*16PK002 multiplication in the rice, the inoculated leaves from both the SA and H_2O pretreatments were collected at 3 DAI, and *Xoo*16PK002 copies were quantified with the qPCR assay. In overall results, the SA-pretreated rice cultivars ('PSL2' and 'PSL2-*Xa21*-BC$_4$F$_6$-L1 and L8') had significantly fewer *Xoo* copies (ranging from 12.8×10^4 to 18.1×10^4 CFU/mL) than the H_2O-pretreated control

(ranging from 25.8×10^4 to 35.7×10^4 CFU/mL) (Figure 5B). Of these, the SA-pretreated rice cultivar 'PSL2' exhibited a significantly lower number of *Xoo* copies (14.0×10^4 CFU/mL) than in the H_2O pretreatment variant (34.7×10^4 CFU/mL), resulting in an approximately 2.4-fold reduction in *Xoo*16PK002 proliferation (Figure 5B). However, the rice cultivar 'IRBB21' in both the SA and H_2O pretreatments showed non-significant differences in the number of *Xoo* copies, i.e. 2.8×10^4 and 2.0×10^4 CFU/mL, respectively (Figure 5B). These results indicated that exogenous SA application in rice cultivars could be a potential strategy against BB disease severity through suppression of *Xoo* proliferation in rice plants.

To evaluate the effect of SA on reducing BB symptoms after *Xoo*16PK002 inoculation in rice, the results revealed that the SA pretreatment (2 mM) significantly reduced the BB severity in the rice 'PSL2', with lesion length on the leaves of 14.1 cm and 41.4 cm at 14 DAI (Figure 5C) and 21 DAI (Figure 5D), respectively, compared to the mock treatment (18.6 cm at 14 DAI and 50.4 cm at 21 DAI). Additionally, the two-introgression lines 'PSL2-*Xa21*-BC$_4$F$_6$-L1 and L8' exhibited moderate BB resistance, with lesion length on the leaves ranging from 10.0 to 10.9 cm at 14 DAI, compared to the H_2O–pretreated control (16.8–18.0 cm) (Figure 5C). Similarly, at 21 DAI, these lines also exhibited moderate BB resistance with lesion lengths ranging from 24.6 cm to 28.0 cm, compared to the H_2O mock pretreatment (34.9–35.1 cm) (Figure 5D). In contrast, both the SA-pretreated rice and H_2O-pretreated rice cultivar 'IRBB21' showed slight differences in the lesion length on the leaves at 14 and 21 DAI (Figure 5C–D). These findings indicated that the SA treatment could significantly reduce symptomatic BB severity in the susceptible rice cultivar 'PSL2' and the introgression lines 'PSL2-*Xa21*', but not in the rice cultivar 'IRBB21'.

In summary, the SA-pretreated rice of two introgression lines 'PSL2-*Xa21*' exhibited reduction in symptomatic BB disease and *Xoo*16PK002 multiplication. However, these lines showed moderate BB resistance to *Xoo*16PK002 than the parental cultivars 'IRBB21' and 'PSL2'.

4. Discussion

The utilization of backcross breeding, along with marker-assisted selection (MAS), is a valuable approach for transferring desired traits from a donor parent into the favored genetic background of a recurrent (or recipient) parent. Additionally, the MAS has proven to be an essential tool in breeding programs, as it enables efficient and cost-effective selection of individual plants carrying desirable genes from large populations (Govintharaj et al., 2018). Based on the mode of gene action and inheritance, the co-dominant marker is more effective in discriminating between homozygous and heterozygous genotypes of individuals than the dominant marker (Collard et al., 2005). In this study, the pTA248 co-dominant marker clearly discriminated three different genotypes (*Xa21/Xa21*; *Xa21/xa21*; and *xa21/xa21*) among the studied rice lines. The pTA248 marker enabled the amplification of a single PCR product with clearly different sizes between the dominant *Xa21* allele and the recessive *xa21* allele, corresponding to 925-bp and 730-bp length, respectively. In agreement with previous findings, the *Xa21*-gene-linked marker (pTA248) was successfully utilized as MAS to identify the *Xa21* gene target in many introgression rice cultivars, such as 'indica RD47' (Suachaowna et al., 2017), 'CSR3' (Baliyan et al., 2018), 'Super Basmati' (Sabar et al., 2019), 'Putra-1' (Chukwu et al., 2019), and 'Ciherang' (Biswas et al., 2021). This finding suggests that the pTA248 co-dominant marker has high accuracy and greatly improved selection efficiency to identify individual plants carrying the desirable gene in the early generations of rice breeding programs.

From previous knowledge, SA is a crucial natural signaling molecule that plays a key role in triggering the activation of the SAR system (Noshi et al., 2016). Additionally, it also activates the SA-dependent signaling pathway by regulating disease resistance mechanisms, such as hypersensitive responses (HR) and defense genes such as *xa5*, *OsNPR1*, and *OsWRKY45* (Jiang et al., 2020), and inhibits pathogen growth (Gális et al., 2004). As shown in Figure 4, the exogenous SA application (at 1 and 2 mM) to the susceptible rice cultivar 'PSL2' significantly suppressed BB-disease progression by exhibiting the shortest lesion length at 7 and 14 DAI of *Xoo*16PK002, compared to

the mock-pretreated control. A similar finding was reported by Shasmita et al. (2019), who observed that exogenous SA treatment (at 2 mM) in the rice cultivar 'Taichung Native 1 (TN1)' provided the most significant reduction in BB disease severity caused by *Xoo* pathogens. More recently, Thepbandit et al. (2021) reported that an optimal concentration range of SA (1–3 mM) conferred the highest efficiency in enhancing BB resistance in the rice cultivar 'KDM105'. This phenomenon suggested that SA functions as an elicitor to induce BB resistance mechanisms. For instance, the SA inducer may be associated with SAR mechanisms, particularly in transmitting defense signals (Suharti & Leana, 2021). Additionally, the SA elicitor may activate HR, leading to cell death at the site of infection (Radojičić et al., 2018), as well as morphological changes, such as increased leaf epidermis thickness and lignin formation in plant tissues (Kavulych et al., 2019).

In this study, the results showed that the SA-pretreated rice cultivar 'IRBB21' and the backcrossed rice lines 'PHS2-*Xa21*-BC$_4$F$_6$' were capable of inducing an earlier and higher expression level of the *Xa21* gene before *Xoo*16PK002 infection (at 0 HAI), compared to the mock pretreatment. However, there is limited evidence to support the hypothesis that exogenous SA application can directly induce *Xa21* gene expression in rice. Only one study has shown that SA signaling positively responded to activate *xa5*-mediated immunity to BB disease in of rice cultivar 'IRBB5', whereas its endogenous SA levels were also especially induced after infection by the *Xoo* strain, PXO86 (Jiang et al., 2020). In contrast, in the H$_2$O-pretreatment conditions, the *Xa21* gene expression was significantly upregulated at a later time point, specifically 2 HAI of *Xoo*16PK002 infection in the rice cultivars 'IRBB21' and the introgression lines 'PSL2-*Xa21*-BC$_4$F$_6$'. This finding was consistent with previous observations that *Xa21* gene expression was up-regulated at 1–2 HAI in rice 'IRBB21' and 'RD47-*Xa21*' (Sagun et al., 2019). Similarly, *Xa21* gene expression has also been shown to confer a higher level of BB resistance with reduced lesion lengths post *Xoo*-inoculated rice harboring *Xa21*-resistance gene lines, compared to susceptible lines (Gao et al., 2018; Hu et al., 2015). This might be explained by the fact that the *Xa21*-mediated immune response is dependent on the presence of bacterial Rax proteins, translated from the Rax operon, which are required for activating *Xa21* gene expression (Pruitt et al., 2015) and promoting BB resistance in rice (Gao et al., 2018).

In this study, the SA-pretreated rice cultivars 'PSL2' and 'PSL2-*Xa21*' were found to effectively suppress the proliferation and growth of *Xoo*16PK002 cells at 3 DAI. Additionally, it was observed that the SA pretreatment led to a reduction in the severity of BB disease caused by *Xoo*16PK002 at 7 and 14 DAI. One explanation was that the SA elicitor could potentially trigger a positive immune response against *Xoo* infection (Jiang et al., 2020). This response could lead to a partial restriction of bacterial *Xoo* proliferation and induce an HR to prevent bacterial spread within rice plants (Radojičić et al., 2018). Moreover, the SA elicitor might be involved in the induction of various immune regulators contributing to plant defense against *Xoo* infection. In agreement with previous findings, the increased SA concentration could trigger rapidly localized cell death (known as the HR) at the specific site of pathogen invasion. This mechanism has proven effective in limiting the symptomatic progression of BB disease (Balint-Kurti, 2019). High levels of SA in rice cultivars ('KDML105', 'IRBB5' or 'IR24') have been shown to strongly reduce *Xoo* multiplication (Thanh et al., 2017; Zhang et al., 2013) at 8 hours post infection (Jiang et al., 2020). Additionally, the regulator of SA signaling (OsNPR1 proteins) enhances the plant defense response by repressing growth and reducing *Xoo* virulence by down-regulating the expression of multiple virulence genes and genes involved in energy metabolism, biosynthesis, and nutrient transportation (Dai et al., 2023). Taken together, these findings indicated that the SA pretreatment with 2 mM in the rice cultivar 'PSL2-*Xa21*' induces up-regulation of the *Xa21* gene and can significantly decrease bacterial *Xoo* proliferation against the symptomatic BB disease in rice.

5. Conclusion

This study aimed to determine the effect of SA application on BB resistance in rice cultivars ('PSL2', 'IRBB21', and backcrossed lines 'PSL2-*Xa21*-BC$_4$F$_6$') after *Xoo*16PK002 inoculation, compared to the mock treatment control. The results

showed that the SA-pretreatment (2 mM) significantly upregulated the *Xa21* gene expression in the rice cultivars 'IRBB21' and 'PSL2-*Xa21*-BC$_4$F$_6$' before *Xoo*16PK002 infection. Furthermore, the SA-pretreatment of the rice led to the suppression of *Xoo*16PK002 proliferation and increased BB disease resistance in the rice cultivars 'IRBB21' and 'PSL2-*Xa21*-BC$_4$F$_6$' after *Xoo*16PK002 inoculation. However, these introgression lines still showed moderate BB resistance to *Xoo*16PK002, compared to the rice cultivar 'IRBB21'. These findings could indicate that BB disease resistance in rice might not only depend on the exogenous SA treatment and the *Xa21* resistance gene but could also be associated with other signaling mechanisms. Thus, the combined functions of SA and *Xa21* should be further studied to gain valuable insights into BB resistance mechanism against *Xoo* invasion in rice.

Data Availability

Data will be provided upon request.

Acknowledgments

References

Balint-Kurti, P. (2019). The plant hypersensitive response: Concepts, control and consequences. *Molecular Plant Pathology*, *20*(8), 1163–1178. https://doi.org/10.1111/mpp.12821

Baliyan, N., Malik, R., Rani, R., Mehta, K., Vashisth, U., Dhillon, S., & Boora, K. S. (2018). Integrating marker-assisted background analysis with foreground selection for pyramiding bacterial blight resistance genes into Basmati rice. *Comptes Rendus Biologies*, *341*(1), 1–8. https://doi.org/10.1016/j.crvi.2017.11.003

Bimolata, W., Kumar, A., Sundaram, R. M., Laha, G. S., Qureshi, I. A., & Ghazi, I. A. (2015). Nucleotide diversity analysis of three major bacterial blight resistance genes in rice. *Plos One*, *10*(3), Article e0120186. https://doi.org/10.1371/journal.pone.0120186

Biswas, P. L., Nath, U. K., Ghosal, S., Goswami, G., Uddin, M. S., Ali, O. M., Latef, A.A.H. A., Laing, A. M., Gao, Y. M., & Hossain, A. (2021). Introgression of bacterial blight Resistance genes in the rice cultivar ciherang: Response against *Xanthomonas oryzae* pv. *oryzae* in the F$_6$ generation. *Plants*, *10*(10), Article 2048. https://doi.org/10.3390/plants10102048

Buddhachat, K., Ritbamrung, O., Sripairoj, N., Inthima, P., Ratanasut, K., Boonsrangsom, T., & Sujipuli, K. (2021). One-step colorimetric LAMP (cLAMP) assay for visual detection of *Xanthomonas oryzae* pv. *oryzae* in rice. *Crop Protection*, *150*, Article 105809. https://doi.org/10.1016/j.cropro.2021.105809

Chukwu, S. C., Rafii, M. Y., Ramlee, S. I., Ismail, S. I., Oladosu, Y., Kolapo, K., Musa, I., Halidu, J., Muhammad, I. I., & Ahmed, M. (2019). Marker-assisted introgression of multiple resistance genes confers broad spectrum resistance against bacterial leaf blight and blast diseases in Putra-1 rice variety. *Agronomy*, *10*(1), Article 42. https://doi.org/10.3390/agronomy10010042

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, *142*, 169–196. https://doi.org/10.1007/s10681-005-1681-5

Dai, X., Wang, Y., Yu, K., Zhao, Y., Xiong, L., Wang, R., & Li, S. (2023). *OsNPR1* enhances rice resistance to *Xanthomonas oryzae* pv. *oryzae* by upregulating rice defense genes and repressing bacteria virulence genes. *International Journal of Molecular Sciences*, *24*(10), Article 8687. https://doi.org/10.3390/ijms24108687

Duan, C., Yu, J., Bai, J., Zhu, Z., & Wang, X. (2014). Induced defense responses in rice plants against small brown planthopper infestation. *The Crop Journal*, *2*(1), 55–62. https://doi.org/10.1016/j.cj.2013.12.001

Duong, C. Q., Bui, A. L., Le, T. H., Tran, T. T., & Trinh, N. N. (2023). Differential biochemical and metabolic responses of contrasting rice cultivars (*Oryza sativa* L.) under salt stress. *Acta Agrobotanica*, *76*, Article 172513. https://doi.org/10.5586/aa/172513

FAOSTAT. (2021). *Crops and livestock products*. Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/#data/QC/visualize

Gális, I., Smith, J. L., & Jameson, P. E. (2004). Salicylic acid-, but not cytokinin-induced, resistance to WClMV is associated with increased expression of SA-dependent resistance genes in *Phaseolus vulgaris*. *Journal of Plant Physiology*, *161*(4), 459–466. https://doi.org/10.1078/0176-1617-01255

Gao, L., Fang, Z., Zhou, J., Li, L., Lu, L., Li, L., Li, T., Chen, L., Zhang, W., Zhai, W., & Peng, H. (2018). Transcriptional insights into the pyramided resistance to rice bacterial blight. *Scientific Reports*, *8*(1), Article 12358. https://doi.org/10.1038/s41598-018-29899-1

Govintharaj, P., Manonmani, S., Karthika, G., & Robin, S. (2021). Introgression of bacterial blight resistance genes (*Xa* 21, *xa* 13 and *xa* 5) into CB 174 R, an elite restorer line in rice. *Biology and Life Sciences Forum*, *4*(1), Article 72. https://doi.org/10.3390/IECPS2020-08759

Govintharaj, P., Manonmani, S., & Robin, S. (2018). Variability and genetic diversity study in an advanced segregating population of rice with bacterial blight resistance genes introgressed. *Ciência e Agrotecnologia*, *42*, 291–296. https://doi.org/10.1590/1413-70542018423022317

Hu, H., Wang, J., Shi, C., Yuan, C., Peng, C., Yin, J., Li, W., He, M., Wang, J., Ma, B., & Wang, Y. (2015). A receptor like kinase gene with expressional responsiveness on *Xanthomonas oryzae* pv. *oryzae* is essential for *Xa21*-mediated disease resistance. *Rice*, *8*, Article 1. https://doi.org/10.1186/s12284-014-0034-1

International Rice Research Institute (IRRI). (2013). *Standard evaluation system for rice* (5th ed.). International Rice Research Institute.

Jiang, G., Yin, D., Shi, Y., Zhou, Z., Li, C., Liu, P., Jia, Y., Wang, Y., Liu, Z., Yu, M., & Wu, X. (2020). OsNPR3.3-dependent salicylic acid signaling is involved in recessive gene *xa5*-mediated immunity to rice bacterial blight. *Scientific Reports*, *10*(1), Article 6313. https://doi.org/10.1038/s41598-020-63059-8

Kavulych, Y., Kobyletska, M., & Terek, O. (2019). Investigation of salicylic acid-induced change on flavonoids production under cadmium toxicity in buckwheat (*Fagopyrum esculentum* Moench) plants. *Eureka: Life Sciences*, *5*, 13–18. https://doi.org/10.21303/2504-5695.2019.00986

Ke, Y., Hui, S., & Yuan, M. (2017). *Xanthomonas oryzae* pv. *oryzae* inoculation and growth rate on rice by leaf clipping method. *Bio-protocol*, *7*(19), Article e2568. https://doi.org/10.21769/BioProtoc.2568

Khoa, N. Đ., Xạ, T. V., & Hào, L. T.. (2017). Disease-reducing effects of aqueous leaf extract of *Kalanchoe pinnata* on rice bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* involve induced resistance. *Physiological and Molecular Plant Pathology*, *100*, 57–66. https://doi.org/10.1016/j.pmpp.2017.06.005

Liang, B., Wang, H., Yang, C., Wang, L., Qi, L., Guo, Z., & Chen, X. (2022). Salicylic acid is required for broad-spectrum disease resistance in rice. *International Journal of Molecular Sciences*, *23*(3), Article 1354. https://doi.org/10.3390/ijms23031354

Liu, F., McDonald, M., Schwessinger, B., Joe, A., Pruitt, R., Erickson, T., Zhao, X., Stewart, V., & Ronald, P. C. (2019). Variation and inheritance of the *Xanthomonas raxX-raxSTAB* gene cluster required for activation of *Xa21*-mediated immunity. *Molecular Plant Pathology*, *20*(5), 656–672. https://doi.org/10.1111/mpp.12783

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Noshi, M., Mori, D., Tanabe, N., Maruta, T., & Shigeoka, S. (2016). *Arabidopsis* clade IV TGA transcription factors, TGA10 and TGA9, are involved in ROS-mediated responses to bacterial PAMP flg22. *Plant Science*, *252*, 12–21. https://doi.org/10.1016/j.plantsci.2016.06.019

Peng, H., Chen, Z., Fang, Z., Zhou, J., Xia, Z., Gao, L., Chen, L., Li, L., Li, T., Zhai, W., & Zhang, W. (2015). Rice *Xa21* primed genes and pathways that are critical for combating bacterial blight infection. *Scientific Reports*, *5*(1), Article 12165. https://doi.org/10.1038/srep12165

Promma, P., Grandmottet, F., & Ratanasut, K. (2016). Characterisation of *Xa21* and defence-related gene expression in RD47 × IRBB21 hybrid rice subject to *Xanthomonas oryzae* pv. *oryzae*. In *Proceedings of the National and International Graduate Research Conference 2016* (pp. 1485–1493).

Pruitt, R. N., Schwessinger, B., Joe, A., Thomas, N., Liu, F., Albert, M., Robinson, M. R., Chan, L. J. G., Luu, D. D., Chen, H., & Bahar, O. (2015). The rice immune receptor *Xa21*

recognizes a tyrosine-sulfated protein from a Gram-negative bacterium. *Science Advances*, *1*(6), Article e1500245. https://doi.org/10.1126/sciadv.1500245

Radojičić, A., Li, X., & Zhang, Y. (2018). Salicylic acid: A double-edged sword for programed cell death in plants. *Frontiers in Plant Science*, *9*, Article 1133. https://doi.org/10.3389/fpls.2018.01133

Sabar, M., Akhter, M., Bibi, T., Riaz, A., Haider, Z., Khan, A. R., & Bibi, A. (2019). Basmati rice lines development carrying multiple bacterial blight resistance genes pyramided using the marker-assisted backcross breeding approach. *Molecular Breeding*, *39*(10-11), Article 155. https://doi.org/10.1007/s11032-019-1047-7

Sagun, C. M. L., Grandmottet, F., & Ratanasut, K. (2019). Differential expression of *Xoo*-induced kinase 1 (*XIK1*), a *Xanthomonas oryzae* pv. *oryzae* responsive gene, in bacterial blight-susceptible and *Xa21*-mediated resistant indica rice cultivars. *Agriculture and Natural Resources*, *53*(4), 334–339. https://doi.org/10.34044/j.anres.2019.53.4.02

Sagun, C. M., Grandmottet, F., Suachaowna, N., Sujipuli, K., & Ratanasut, K. (2020). Validation of suitable reference genes for normalization of quantitative reverse transcriptase-polymerase chain reaction in rice infected by *Xanthomonas oryzae* pv. *oryzae*. *Plant Gene*, *21*, Article 100217. https://doi.org/10.1016/j.plgene.2019.100217

Schwessinger, B., & Ronald, P. C. (2012). Plant innate immunity: Perception of conserved microbial signatures. *Annual Review of Plant Biology*, *63*, 451–482. https://doi.org/10.1146/annurev-arplant-042811-105518

Shasmita, M. D., Mohapatra, P. K., Naik, S. K., & Mukherjee, A. K. (2019). Priming with salicylic acid induces defense against bacterial blight disease by modulating rice plant photosystem II and antioxidant enzymes activity. *Physiological and Molecular Plant Pathology*, *108*, Article 101427. https://doi.org/10.1016/j.pmpp.2019.101427

Suachaowna, N., Grandmottet, F., Sanyong, S., Palawisut, S., Sujipuli, K., & Ratanasut, K. (2017). The *Xa21* in the backcross introgression lines, BC_4F_2, derived from the Thai rice cultivar 'RD47'/'IRBB21' cross enhances the bacterial blight resistance against *Xanthomonas oryzae* pv. *oryzae* newly isolated from Phitsanulok province. In *The 13th Asian Congress on Biotechnology 2017 (ACB 2017) "Bioinnovation and Bioeconomy"*.

Suharti, W. S., & Leana, N. W. A. (2021). Induce resistance of rice plants against bacterial leaf blight by using salicylic acid application. *IOP Conference Series: Earth and Environmental Science*, *746*(1), Article 012003. https://doi.org/10.1088/1755-1315/746/1/012003

Taratima, W., Samattha, C., Maneerattanarungroj, P., & Trunjaruen, A. (2023). Physiological and anatomical response of rice (*Oryza sativa* L.) 'Hom Mali Daeng' at different salinity stress levels. *Acta Agrobotanica*, *76*, Article 764. https://doi.org/10.5586/aa.764

Thanh, T. L., Thumanu, K., Wongkaew, S., Boonkerd, N., Teaumroong, N., Phansak, P., & Buensanteai, N. (2017). Salicylic acid-induced accumulation of biochemical components associated with resistance against *Xanthomonas oryzae* pv. *oryzae* in rice. *Journal of Plant Interactions*, *12*(1), 108–120. https://doi.org/10.1080/17429145.2017.1291859

Thepbandit, W., Papathoti, N. K., Daddam, J. R., Thumanu, K., Siriwong, S., Thanh, T. L., & Buensanteai, N. (2021). Identification of salicylic acid mechanism against leaf blight disease in *Oryza sativa* by SR-FTIR microspectroscopic and docking studies. *Pathogens*, *10*(6), Article 652. https://doi.org/10.3390/pathogens10060652

Yan-chang, L., Shou-hai, W., Cheng-quan, L., Shuang, W., De-zheng, W., & Shi-yun, D. (2004). Improvement of resistance to bacterial blight by marker-assisted selection in a wide compatibility restorer line of hybrid rice. *Rice Science*, *11*(5-6), Article 231.

Yasmin, S., Hafeez, F. Y., Mirza, M. S., Rasul, M., Arshad, H. M., Zubair, M., & Iqbal, M. (2017). Biocontrol of bacterial leaf blight of rice and profiling of secondary metabolites produced by *rhizospheric Pseudomonas aeruginosa* BRp3. *Frontiers in Microbiology*, *8*, Article 1895. https://doi.org/10.3389/fmicb.2017.01895

Zhang, F., Du, Z., Huang, L., Cruz, C. V., Zhou, Y., & Li, Z. (2013). Comparative transcriptome profiling reveals different expression patterns in *Xanthomonas oryzae* pv. *oryzae* strains with putative virulence-relevant genes. *Plos One*, *8*(5), Article e64267. https://doi.org/10.1371/journal.pone.0064267