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Research article

Effects of nitrogen levels on sucrose content, disease severity of *Xanthomonas oryzae* pv. *oryzae* and yield of hybrid rice (BC_4F_5)

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Abstract

Importance of the work: Phitsanulok 2 (PSL2) is a high-yielding rice commonly cultivated in lower northern Thailand. However, it is susceptible to bacterial blight (BB) disease caused by the bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), resulting in major grain yield losses.

Objectives: To investigate the BB resistance levels and the effect of nitrogen levels on the sucrose contents in BC_4F_5 lines carrying the *Xa21* gene.

Materials & Methods: The *Xa21* gene was introduced into PSL2 using marker-assisted backcrossing. The five BC_4F_5 lines carrying *Xa21* and parental lines were grown with different nitrogen levels, followed by inoculation with *Xoo*E.

Results: All five BC_4F_5 lines had the highest resistance to the BB disease at 75 kg N/ha. The lesion lengths of plants treated with 75 kg N/ha, 150 kg N/ha and 225 kg N/ha under greenhouse conditions were in the ranges 2.8–3.9 cm, 5.65–6.22 cm and 7.67–8.25 cm, respectively. In the field experiment, diseased leaf areas were in the range 7.63–8.34% in the BC_4F_5 lines, which were not significantly different to IRBB21. The sucrose content in the rice leaves increased when a higher rate of nitrogen fertilizer was applied. All BC_4F_5 lines had similar plant heights to PSL2. Three BC_4F_5 lines had greater grain yields per plant than PSL2.

Main finding: All BC_4F_5 lines had greater yields and showed similar resistance to BB as IRBB21 under field trails at 75 kg N/ha. This study revealed that an overdose of nitrogen fertilizer enhanced the sucrose content in rice leaves and induced severe BB disease.

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Introduction

Rice (*Oryza sativa* L.) is an important economic crop for both domestic consumption and export to the world market; in 2018–2019 northern Thailand had a cultivated area of 2.21 million ha, yielding 7.84 million t of paddy, with an average yield of 3.4 t/ha (Office of Agricultural Economics, 2019), which is considered to be a relatively low yield. This low yield is influenced by invasions of diseases and pests of rice, such as bacterial blight (BB) disease caused by the bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), which has been found in epidemic proportions in irrigated fields globally, as well as being one of the most prevalent diseases in lower northern Thailand (Mew, 1987). Infestation of this pathogen results in a 20–50% reduction in rice yields (Ou, 1985) but can be up to 80% when the epidemic is severe (Perumalsamy et al., 2010). Over-application of nitrogen fertilizer will exacerbate disease incidence, as the fertilizer results in increased leaf growth that is very succulent and hence more susceptible to certain diseases (Reddy, 1979). In 2016, the incidence of leaf blight disease was quite high after applying increased nitrogen fertilizer rates (Sharma, 2016). Experimental results showed that the low amount of nitrogen fertilizer (25 kg/ha) had the lowest effect on the severity of the disease, while it decreased yield (Chaudhary et al., 2009).

Phitsanulok 2 (PSL2) is an indica steamed rice not sensitive to photoperiod (Rice Research and Development Bureau, 2020). It is a popular rice cultivar for cultivation in lower northern Thailand and has a harvest age of 119–121 d and is resistant to brown planthopper, white-backed planthopper and green leafhoppers, although it is sensitive to rice blast disease and rice ragged stunt disease and is susceptible to BB (Rice Research and Development Bureau, 2020).

More than 38 BB-related genes have been identified in rice, with many of them being used in rice breeding programs for BB resistance (Khan et al., 2014). In Thailand, the bacterial blight resistance *Xa4*, *Xa7*, *xa5* and *xa13* genes were reported to exit in 155 Thai rice cultivars (Sombunjitt et al., 2017). The International Rice Research Institute had developed several near-isogenic lines (NILs) with BB resistance genes using an IR24 genetic background, such as IRBB1, IRBB2, IRBB3, IRBB4, IRBB5, IRBB7, IRBB10, IRBB11 and IRBB21. Among these resistance genes, *Xa21* has been wildly studied (Song et al., 1997; Perez et al., 2008). An *Xa21* gene was the first successfully cloned receptor kinase-like protein gene from a wild African species *O. longistaminata* (Song et al., 1995), and *Xa21* was transferred into IR24, resulting in the near-isogenic line, IRBB21. Many studies reported that IRBB21 is resistant to many *Xoo* strains (Khush et al., 1989; Swamy et al., 2006; Perez et al., 2008; Sagun et al., 2018). Thus, the objectives of the current study were to improve the BB resistance level of PSL2 using marker-assisted backcrossing and to investigate the effect of nitrogen fertilizer rates and sucrose content on disease resistance. The data obtained from this study will help assess guidelines for the BB-resistant development of fine rice cultivars susceptible to bacterial blight disease.

Materials and Methods

Plant materials

The NIL IRBB21 carrying *Xa21* was used as the BB resistant donor. The F_1 plants were derived from a cross between PSL2 as the recipient parent and IRBB21; then, the F1 hybrids were backcrossed to PSL2 four times to generate the BC_4F_1 generation. The BC_4F_2 population was derived from individual self-pollinated BC_4F_1 plants. The selected BC_4F_2 lines were self-pollinated to obtain BC_4F_3 seeds. The selected BC_4F_3 lines were self-pollinated to generate the BC_4F_5 population. Five selected BC_4F_5 lines were used in this study. The susceptible check used in this study was PSL2.

Xanthomonas oryzae pv. oryzae (Xoo) inoculum preparation and inoculation

Testing for bacterial blight resistance in rice populations used *Xanthomonas oryzae* pv. *oryzae* isolate *Xoo*E provided by courtesy of the Center of Excellence in Research for Agricultural Biotechnology, Naresuan University, Phitsanulok, Thailand. The isolate was inoculated on rice plants using the clipping method (Kauffman et al., 1973). Bacteria for inoculation were prepared by growing on nutrient agar medium that was incubated at 28 ± 2 °C for 72 h until the germ had grown into a single colony. The single colony of *Xanthomonas oryzae* pv. *oryzae* was further sub-cultured in liquid nutrient broth medium at 28 ± 2 °C for 72 h and the cell suspension was diluted into 1×10^8 cells/ mL of distilled water, which was confirmed by measurement using a spectrophotometer (Boeco model S-200 VIS & S-220 UV/VIS; Hamburg, Germany) with A_{600} optical density. This sub-culture was used to inoculate the tested plants grown in the greenhouse and in the field.

Greenhouse screening

The greenhouse experiment using different nitrogen rates was conducted in climate greenhouse system with a non-controlled environment, using natural light and temperature with high humidity simulation based on artificial rainfall during inoculation. The experimental layout used a randomized complete block design with three replicates, consisting of three different levels of fertilizer (N-P-K): 75–37.5–75 (N75), 150–37.5–75 (N150) and 225–37.5–75 (N225) kg/ha, and seven rice cultivars: PSL2, IRBB21, PSL2-*Xa21* L.2, PSL2-*Xa21* L.3, PSL2-*Xa21* L.4, PSL2-*Xa21* L.5 and PSL2-*Xa21* L.8. Surface-sterilized seeds were soaked in water at 37 °C for 24 h. Germinated seedlings were transferred to a seedling tray. At day 14 after sowing, the seedlings were transferred into plastic pots that each contained 5 kg of farm soil with one plant per pot. The fertilization was divided into two parts equally. The fertilizers were applied to the plants at 30 d and 60 d after transplanting. All plants were inoculated with *Xoo*E at the tillering stage by clipping 2–3 cm from the tip of the five youngest fully expanded leaves.

Evaluation of agronomical traits and grain yield

A field screening experiment was conducted using a randomized complete block design with three replicates consisting of seven rice cultivars: PSL2, IRBB21, PSL2-*Xa21* L.2, PSL2-*Xa21* L.3, PSL2-*Xa21* L.4, PSL2-*Xa21* L.5 and PSL2-*Xa21* L.8. Based on the results obtained from the greenhouse test, a recommended dose of fertilizers at 75–37.5–75 (N–P–K) kg/ha was applied, due to the low infestation of bacterial blight and the aim of promoting the rice plants to be more resistant to BB. Surface-sterilized seeds were soaked in water at 37 °C for 24 h. Germinated seedlings were transferred to a seedling tray. Then seedling aged 21 d were transplanted maintaining 30 cm × 30 cm for the plant-to-plant and row-to-row distances. The agronomical traits were observed at the Naresuan University site, planted during the monsoon season (July–October 2021). Fertilization was applied at two times in equal amounts, with the first applied at 7 d after transplanting. The second application occurred at 55 d after planting. All plants were inoculated with *Xoo*E at the tillering stage (60 d after transplanting) by clipping 2–3 cm from the tips of the five youngest, fully expanded leaves. Agronomic performance was evaluated based on measuring nine traits: plant height, number of tiller per plant, number of panicles per plant, days to (50%) flowering, days to harvesting, panicle length, number of filled grains, 100-grain weight and grain weight per plant. Grain weight was evaluated at 14% moisture content.

Genotype characterization

The genotypes in the NILs were examined using the ready-made Phire® direct plant polymerase chain reaction (PCR) kits (Thermo Fisher Scientific; USA). The marker used for determining the presence of *Xa21* gene was pTA248. The base sequence of the pTA248 primer pairs was: pTA248-Forward 5'-AGACGCGGAAGGGTGGTTCCCGGA-3' and pTA248-Reverse 5'-AGACGCGGTGTAATCGAAAGATG AAA-3' (Shanti et al., 2010). The PCR product of the *Xa21* gene was obtained from the PCR reaction using an electrophoresis technique with 1.2% agarose gel concentration. DNA was separated using a 100 V electromotive force for 50 min in 1X TAE buffer. RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology, South Korea) was used.

Evaluation of pathogenicity of Xanthomonas oryzae pv. Oryzae

Pathogenicity was assessed from cut marks formed on the rice leaves at 14 d after inoculation. Plants were characterized as resistant or susceptible based on lesion lengths under greenhouse conditions (Table 1) and under field conditions (Table 2), according to the International Rice Research Institute (IRRI) Standard Evaluation System for BB resistance (International Rice Research Institute, 1996). The disease index under field conditions was calculated using Equations 1 and 2:

$$\text{Disease index (\%)} = \frac{\text{Number of bacterial leaf blight infected plants}}{\text{Total number of plants examined}} \times 100 \quad (1)$$

$$\text{Disease index (\%)} = \frac{n(0) + n(1) + n(3) + n(5) + n(7) + n(9)}{t(n)} \times 100 \quad (2)$$

where n(0), n(1), n(3), n(5), n(7) and n(9) are the numbers of leaves showing severity scores of 0, 1, 3, 5, 7 and 9, respectively, according to Table 2 and t(n) is the total number of leaves scored (McKinney, 1923).

Table 1 Standard Evaluation System for bacterial blight (BB) resistance in the greenhouse infection test. (International Rice Research Institute, 1996)

Lesion Length (cm)	Level of BB resistance
0–5	Resistant (R)
> 5–10	Moderately resistant (MR)
> 10–15	Moderately susceptible (MS)
> 15	Susceptible (S)

Table 2 Standard Evaluation System for bacterial blight (BB) resistance in the field infection test. (International Rice Research Institute, 1996)

Scale	Diseased leaf area (%)	Level of BB resistance
1	1–5	Resistant (R)
3	6–12	Moderately resistant (MR)
5	13–25	Moderately susceptible (MS)
7	26–50	Susceptible (S)
9	> 50	Highly susceptible (HS)

Analysis of sucrose content

The youngest fully expanded leaves of an individual plant were used to observe the sucrose content by weighing 0.1 g fresh, followed by crushing with liquid nitrogen. Then, 1 mL of distilled water was added and the samples were incubated at 65 °C for 20 min. The samples were centrifuged at 14,000 revolutions per minute for 5 min. The translucent extract was added to a Megazyme sucrose/d-glucose assay kit (Megazyme, Ireland), divided into two parts (A and B). Part A is for detecting free D-glucose, while part B is for free D-glucose plus D-glucose from sucrose. The absorbance was measured at a wavelength of 510 nm using a spectrophotometer (Boeco model S-200 VIS & S-220 UV/VIS; Hamburg, Germany), and the sucrose content in grams per liter of sample solution was calculated using the formula:

$$\text{Sample solution (g/L)} = (\Delta B - \Delta A) * F * \text{Dilution} * 0.0095$$

where ΔB is the absorbance of free D-glucose plus D-glucose from sucrose, ΔA is the absorbance of free D-glucose, F is the factor to convert from absorbance to micrograms for 100 µg D-glucose (= 100 / absorbance for 100 µg D-glucose) and Dilution is the dilution of the original sample solution.

Then, the sucrose content in milligrams per gram was calculated using the formula:

$$\text{Sucrose} = (A) / \text{Weight}_{\text{sample}}$$

where the $\text{Weight}_{\text{sample}}$ is measured in grams per liter of sample solution.

Statistical analysis

Data were analyzed using one-way analysis of variance. Duncan's multiple range test at the 95% confidence level ($p < 0.05$) was used to compare differences between treatments. Statistical analysis was performed using the R software package (R Core Team, 2021).

Results and Discussion

Genotype characterization

The pTA248 primer is a co-dominant molecular marker that can be used to detect three genotypes: homozygous *Xa21* genes, heterozygous *Xa21* genes and without *Xa21* genes. The pTA248 primer provided a PCR product of 925 bp and 730 bp in rice samples with and without *Xa21* gene, respectively. The results showed that IRBB21-resistant rice contained the *Xa21* gene, with the near-isogenic line PSL2-*Xa21* containing the *Xa21* gene in homozygous condition, in which the PSL2 cultivar was susceptible to bacterial blight without the *Xa21* gene, as shown in Fig. 1.

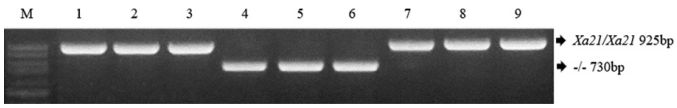

Fig. 1 Banding pattern of genotypes of three rice cultivars showing presence and absence of *Xa21* gene, where polymerase chain reaction products amplified by pTA248 primers revealed 925 and 730 bp size fragments, lanes 1–3 = PSL2-*Xa21*, lanes 4–6 = PSL2 and lanes 7–9 = IRBB21, respectively and M represents 100 bp DNA ladder

Evaluation of the pathogenicity, Xanthomonas oryzae pv. oryzae

From the assessment of the pathogenicity of the germ pathogens in all rice cultivars, it was found that the different levels of nitrogen fertilizer influenced the pathogenesis of the disease at 14 d after inoculation. The resistance to BB disease was highly significantly different ($p \leq 0.001$). The PSL2 cultivar showed very weak resistance to the BB disease at all nitrogen fertilizer levels. The disease incidence was high at the fertilizer level of 75 kg/ha and the highest at the fertilizer level of 225 kg N/ha. IRBB21 showed high resistance to BB disease at all nitrogen fertilizer levels, as shown in Table 3 and Fig. 2. All BC_4F_5 lines showed resistance to BB disease at 75 kg/ha of nitrogen fertilizer. The lesion length increased when the nitrogen fertilizer level increased. This result was consistent with other studies, which reported that the incidence of leaf margin disease was greater with increased nitrogen fertilizer rates and the lesion length was significantly different (Sharma, 2016). In addition, the different response in disease resistance that was influenced by the different nitrogen fertilizer rates measured in the current study was consistent with Khan et al. (2014) who reported that different rice varieties may differ in their disease resistance mechanisms. It is possible that each type of rice has different genes for resistance to pathogens. It was also found that the environment affected the expression of resistance genes.

Table 3 Disease development, lesion length (cm), on two parental lines and five near-isogenic lines at 14 d after inoculation by *Xanthomonas oryzae* pv. *oryzae* strain *Xoo*E grown under three nitrogen levels under greenhouse conditions

N level	PSL2		IRBB21		PSL2-*Xa21*L.2		PSL2-*Xa21*L.3		PSL2-*Xa21*L.4		PSL2-*Xa21*L.5		PSL2-*Xa21*L.8	
(kg/ha)	Mock	Infection	Mock	Infection	Mock	Infection	Mock	Infection	Mock	Infection	Mock	Infection	Mock	Infection
75	0.15±0.01	24.71±0.3^{c}	0.17±0.02	1.69±0.06^{c}	0.23±0.02	3.90±0.09^{c}	0.21±0.01	3.40±0.21^{c}	0.22±0.04	4.0±0.86^{c}	0.21±0.11	2.80±0.05^{c}	0.23±0.04	3.80±0.74^{c}
150	0.14±0.01	28.03±0.31^{b}	0.21±0.01	2.33±0.15^{b}	0.31±0.02	6.07±0.19^{b}	0.25±0.01	5.65±0.24^{b}	0.27±0.01	6.22±0.54^{b}	0.24±0.03	5.85±0.52^{b}	0.28±0.12	6.02±0.48^{b}
225	0.16±0.01	37.59±0.92^{a}	0.19±0.02	2.99±0.11^{a}	0.2±0.01	8.07±0.22^{a}	0.3±0.01	8.17±0.44^{a}	0.25±0.03	8.42±0.72^{a}	0.26±0.02	7.67±0.74^{a}	0.28±0.11	8.25±0.21^{a}
CV%	34.7	4.35	13.15	10.14	37.73	17.85	34.43	20.25	26.12	16.45	29.14	14.1	31.24	18.85
F-test	ns	***	ns	***	ns	***	ns	***	ns	***	ns	***	ns	***

*** = highly significant differences of means among treatments at $p < 0.001$; ns = non-significant difference
Mean values (± SD) in each column superscripted with different lowercase letters are significantly ($p < 0.05$) different

Fig. 2 Severity of bacterial blight disease on all rice lines at 14 d after inoculation with *Xanthomonas oryzae* pv. *oryzae* under greenhouse conditions, where levels of lesion length on rice leaves indicate moderate resistant (MR) and susceptible (S).

The field screening results showed that all BC_4F_5 lines had the same resistance score (MR) as the resistance line (IRBB21), while PSL2 was highly susceptible (HS) to BB disease, as shown in Table 4 and Fig. 3. There was greater infection in the paddy field than under the greenhouse conditions. PSL2 produced a higher infection rate (61%) than IRBB21 (7%) and all BC_4F_5 lines (7–8%). The results obtained through the multi trials indicated that the landrace cultivar lacking resistant genes was susceptible to BB disease. The disease occurrence in the paddy field was higher than in the non-controlled climate greenhouse system because the field experiment was conducted during the rainy season (July–October 2021), when the rainfall, wind speed, optimal temperature and high humidity could induce severe infection in the plants in the field trial. The non-controlled climate greenhouse system used in this study was surrounded with nylon netting to protect insects from entering due to the higher temperature and humidity inside the greenhouse during the summer season than outside. The optimal temperature and high humidity substantially affected infection under the field conditions, resulting in the severity

Fig. 3 Characteristics of rice cultivars after inoculation with *Xanthomonas oryzae* pv. *oryzae* in field test at: (A) 0 d; (B) 14 d, where A = PSL2, B = IRBB21, C = PSL2-*Xa21* L. 8, D = PSL2-*Xa21* 5, E = PSL2-*Xa21* 4, K = PSL2-*Xa21* L.3 and G = PSL2-*Xa21* L. 2

Table 4 Disease reaction score of parental lines and BC_4F_5 lines for bacterial blight (BB) under field conditions at 75 kg N/ha

Cultivar	*Xa* gene	Diseased leaf area (%)	Response
PSL2	-	61.67±1.67[a]	HS
IRBB21	*Xa21/Xa21*	7.67±0.34[b]	MR
PSL2-*Xa21* L.2	*Xa21/Xa21*	8.00±0.00[b]	MR
PSL2-*Xa21* L.3	*Xa21/Xa21*	7.67±0.67[b]	MR
PSL2-*Xa21* L.4	*Xa21/Xa21*	8.34±0.34[b]	MR
PSL2-*Xa21* L.5	*Xa21/Xa21*	8.34±0.34[b]	MR
PSL2-*Xa21* L.8	*Xa21/Xa21*	7.34±0.34[b]	MR
p Value		≤ 0.001	
Coefficient of variation (%)		8.06	

MR = moderately resistant; HS = highly susceptible (HS)

Mean± SD (n = 3) in a column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

of BB and enhancement of the breakdown of the plant disease resistance genes (Webb et al., 2010). On the other hand, the high temperature and high humidity in the greenhouse cloud reduced the infection of BB. Therefore, if the given greenhouse conditions were not suitable; they may not have adequately mimicked the complexities of natural disease infection, causing limited disease infection. As indicated in this study, all five NILs expressed moderate resistance to *Xoo*E in the field trial. The similar resistance to BB in these five selected BC_4F_5 lines may indicate that the genetic background required for the *Xa21*-mediated BB resistance in the BC_4F_5 population was stable, as most genes involved in this resistance had a high level of homozygosity.

Based on this finding, the different responses by the rice varieties to bacterial *Xoo* were caused by genetic variation and the nitrogen fertilizer rates. Rice varieties carrying the *Xa21* gene (IRBB21 and the near-isogenic PSL2-*Xa21* lines) showed resistant response to the *Xoo*E strain which was virulent to the susceptible check cultivar (PSL2). Similar results were also reported that rice cultivars carrying *Xa21* could induce an effective defense response to multiple *Xoo* strains (Song et al., 1995). This was interesting because rice cultivars with the *Xa21* gene were more resistant than *Xa21*-free cultivars. Rice plants applied with the high-N concentration generally exhibited higher disease severity. The same result was also confirmed by Manzoor et al. (2017) that deficient and excessive uses of N fertilization increased BB disease severity. Furthermore, the variation of BB disease incidence with different planting locations and times indicated that weather conditions play an important role in BB incidence. It has been reported that rainfall and humidity are the major important factors affecting the development and infection of BB disease (Gopalakrishnan et al., 2008).

Analysis of sucrose content

The analysis of the sucrose content in the rice leaves revealed that the different levels of nitrogen fertilizers influenced the sucrose levels in all plants. Increasing the nitrogen fertilizer rates increased the sucrose content in the control plants. At 14 d after inoculation, the sucrose content decreased compared to the experiment in which the leaves were cut with distilled water (mock), as shown in Fig. 4. The leaves that were treated with sterile distilled water had higher

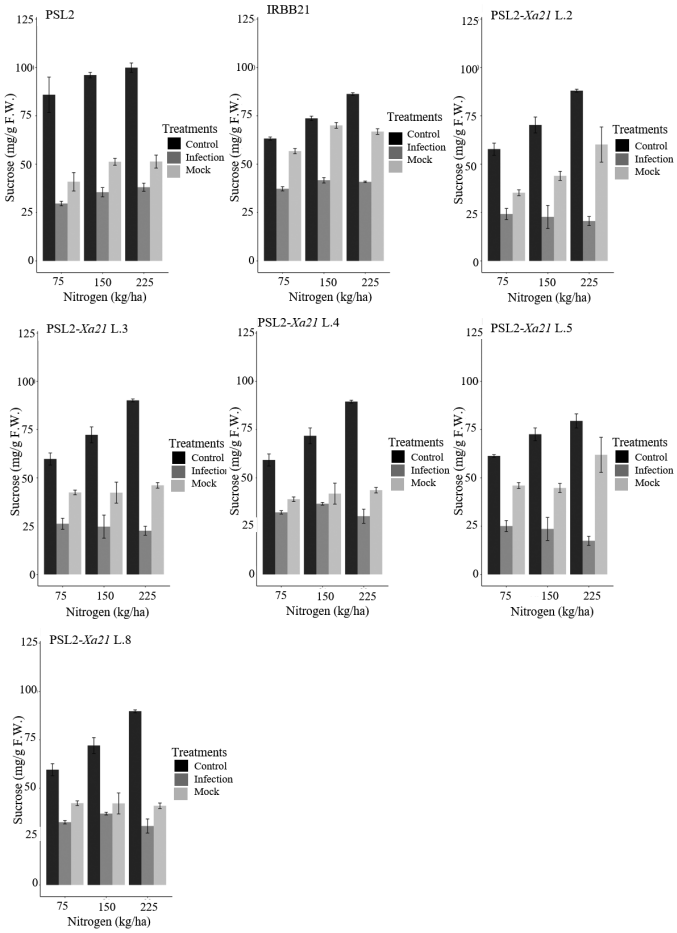

Fig. 4 Sucrose content in rice leaves of all rice cultivars under different nitrogen concentrations, where gray color = *Xanthomonas oryzae* pv. *oryzae* infected leaf, each data point represents mean ± SD from three plants and F.W. = fresh weight

sucrose levels than the inoculated leaves. These phenomena were the same in all rice cultivars, indicating that the BB pathogen grows and invades the leaves rapidly when there is a high sucrose concentration in the leaves. After 14 d of infestation of *Xoo*E, the level of sucrose in the leaves was significantly reduced, showing that the pathogen used sucrose as a source of nutrient for its own growth, resulting in the disease spreading rapidly in plants where a high nitrogen level had been applied. This was consistent with research that showed sucrose is produced in the mesophyll cells and transported via the phloem cells throughout the plant, with the sucrose being released from cells in higher amounts, thereby making it easier for pathogens to invade (Chen et al., 2012). Nitrogen is derived through a sugar transport mechanism in plants (Wu et al., 2018), where low levels of sucrose contribute to low disease susceptibility (Wu et al., 2019). Nitrogen plays a key role in promoting growth and stimulating plants to increase tillering, thus affecting the number of spikes per area, as well as increasing the number of seeds per spike. Over-fertilization by nitrogen application increased the incidence of disease. Therefore, considering the yield and cost of fertilizing, it should not be applied more than is necessary.

Evaluation of yield performance of five BC_4F_5 lines

Based on agronomic performance, five BC_4F_5 lines were selected as promising lines for establishing PSL2 with BB resistance (Table 5). All nine examined traits of the promising line were very similar to those of the recurrent parent. Plant height ranged from 124 cm (PSL2-*Xa21* L.8) to 127 cm (PSL2-*Xa21* L.5), compared to 126 cm for the recurrent parent, PSL2. The number of tillers per plant ranged from 18 (PSL2-*Xa21* L.4) to 23 (PSL2-*Xa21* L.2), compared to 19 for the recurrent parent, PSL2. The number of filled grains per plant ranged from 379 (PSL2-*Xa21* L.3) to 466 (PSL2-*Xa21* L.2), compared to 428 for the recurrent parent, PSL2. The grain weight per plant ranged from 49 g (PSL2-*Xa21* L.5) to 93 g (PSL2-*Xa21* L.2), compared to 50 g for the recurrent parent, PSL2. In general, it required six to eight backcross cycles to obtain the background of the recurrent parent. However, in the earlier generation (after BC_2) it may be possible to distinguish between backcross progeny and the recurrent parent for individual plants (Hasan et al., 2015; Nan et al., 2019).

Conclusion

BB-resistant PSL2 lines were successfully developed by introgressing *Xa21* through marker-assisted selection (MAS). The phenotypic background selection implemented during MAS was effective and provided rapid agronomic performance screening of the PSL-*Xa21* original lines. The greenhouse screening showed a clear relationship between an overdose of the nitrogen level and the sucrose content that was related to BB severity in both susceptible and resistant cultivars. The field evaluation of all PSL-*Xa21* BC_4F_5 lines demonstrated that selected lines had equivalent agro-morphological and yield traits for BB. This higher level of resistance to BB disease observed among the five NILs without yield penalty should be a positive outcome for farmer struggling with BB disease in Thailand. Therefore, more trails with further backcross lines and various *Xoo* strains with different virulent genes should provide more important information on the durability and reliability of the BB-resistant hybrid for Thai farmers.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Table 5 Agronomic traits and yield of five BC_4F_5 (NILs) lines averaged from three individuals of each line in 2021 monsoon season

Cultivar	Plant height (cm)	Tillers per plant (cm)	Panicles per plant (panicle)	Days to flowering (d)	Days to harvesting (d)	Panicle length (cm)	Filled grains (grains)	100-grain weight (g)	Grain weight per plant (g)
PSL2	126.67±1.86^{a}	19.67±1.46	19±3.22	83±0.12	114±0^{a}	30.84±0.45^{a}	428±51.82^{ab}	3.39±0.2^{ab}	50.86±5.52^{b}
IRBB21	118±0.58^{b}	22±3.61	21±2.31	83±0.06	114±0^{a}	28.5±0.29^{b}	551.34±49.75^{a}	2.84±0.0^{4b}	76.2±21.58^{ab}
PSL2-*Xa21* L.2	124.67±0.34^{a}	23.67±1.77	17.67±1.46	82±0.12	112±0^{b}	31±0.58^{a}	466.34±15.6^{ab}	3.51±0.1^{a}	93.02±3.58^{a}
PSL2-*Xa21* L.3	125.34±0.89^{a}	21±1.53	17±2.01	81.67±0.34	112±0^{b}	30.34±0.73^{a}	379.67±22.46^{b}	3.51±0.17^{a}	64.52±1.38^{b}
PSL2-*Xa21* L.4	124.67±1.46^{a}	18.32±0.34	15.34±1.21	81.67±0.34	112±0^{b}	30±0.58^{ab}	449.67±19.06^{ab}	3.48±0.31^{a}	50.54±1.98^{b}
PSL2-*Xa21* L.5	127.67±0.89^{a}	18.34±1.21	17.67±1.21	81.34±0.89	112±0^{b}	29.67±0.34^{ab}	437±55.9^{ab}	3.16±0.06^{ab}	49.73±5.45^{b}
PSL2-*Xa21* L.8	124.34±2.97^{a}	22±1.16	18.34±0.89	81.67±0.89	112±0^{b}	29.84±0.45^{ab}	460±23.72^{ab}	3.31±0.26^{ab}	61.9±4.04^{b}
p-value	0.0136	0.352	0.556	0.168	≤ 0.001	0.049	0.047	0.045	0.033
CV%	2.13	15.31	18.35	1.07	0	2.91	14.42	9.7	24.29

DTF = days to 50% flowering; DTH = day to harvesting

Mean (± SD) in each column superscripted with different lowercase letters are significantly ($p < 0.05$) different

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