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Precise Genotype Selection in 'PSL2-*Xa21*' Rice Introgression Lines Using Whole Genome Sequencing

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Abstract

Rice bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), poses a major threat to global rice production. Enhancing BB resistance through gene introgression is a sustainable strategy for disease management, particularly in elite cultivars like 'Phitsanulok2' ('PSL2'), which has high yield potential and insect resistance but is susceptible to BB. This study improved BB resistance in 'PSL2' by introgressing the *Xa21* gene, a broad-spectrum resistance gene from the donor parent 'IRBB21' through backcross breeding combined with whole genome sequencing (WGS)-assisted selection. Three BC5F6 introgression lines were evaluated for agronomic performance and BB resistance under field conditions. These lines exhibited increased plant height and grain yield compared to both parents, with no significant differences in tiller numbers. Lines 'A' and 'B' showed moderate resistance (MR) to BB, while line 'C' demonstrated resistance (R). WGS revealed that seven of the nine BC5F6 lines retained 71.78%–73.31% genomic similarity with the recurrent parent 'PSL2'. Seventeen *Xa21*-mediated immune response genes were identified, highlighting their potential contributions to BB resistance. Genomic composition analysis showed substantial recovery of the recurrent parent genome, with notable donor genome segments retained on chromosome 11 and heterozygous regions observed on chromosomes 3, 6, 7, 8, and 9. Subsequent evaluation of BC5F7 lines under greenhouse conditions confirmed significant improvements in plant height, tiller number, and grain yield with moderate BB resistance in seven of the nine lines. Results demonstrated the effectiveness of combining advanced backcross breeding with WGS-assisted selection to develop high-yielding, BB-resistant rice lines. The identification of immune-related genes and their interactions offers valuable insights for optimizing future rice breeding strategies.

Keywords: Backcross Breeding; Bacterial Blight; Genetic Composition Analysis; Rice Resistance Gene; *Xanthomonas oryzae pv. oryzae* (Xoo).

1. Introduction

Rice (*Oryza sativa* L.) is a major staple crop, providing sustenance for more than half of the global population [1, 2]. Asia dominates rice production, with Thailand ranking as the 6th largest producer (accounting for 4% of global production) and the 3rd largest exporter (13% of global exports) [3, 4]. In Thailand, the rice variety 'Phitsanulok2'

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('PSL2'), developed by the Phitsanulok Rice Research Center, is widely cultivated in the lower northern region due to its high yield potential, superior grain quality, and resistance to major insect pests such as the brown planthopper and green rice leafhopper. However, despite these advantages, 'PSL2' remains highly susceptible to bacterial blight (BB), a serious threat to rice production caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) [5, 6], which is frequently found in this region [7].

BB is among the most destructive bacterial diseases in rice, causing yield losses ranging from 30–60% [8–10] and, in severe cases, up to 80–90%, depending on the rice variety, stage of plant development, the virulence of the *Xoo* strain, and environmental conditions [11, 12]. Current management strategies, including the use of antagonistic bacteria [13], cultural practices [5], and chemical treatments [14], offer limited and often inconsistent efficacy. These approaches are constrained by high costs, environmental concerns, and potential health risks. Consequently, breeding to develop BB resistance through the introgression of resistant (*R*) genes into susceptible but agronomically superior cultivars has emerged as a cost-effective, environmentally sustainable, and durable disease management strategy [6, 15–17].

To date, 46 *R* genes conferring resistance to BB have been identified at the molecular level [16, 18]. Among these, the dominant *Xa21* gene, originally derived from *Oryza longistaminata*, has been widely used in breeding programs due to its broad-spectrum and race-specific resistance [19]. *Xa21* encodes a receptor-like protein kinase (RLK) that detects pathogen-associated molecular patterns (PAMPs), initiating immune responses upon pathogen recognition [20, 21]. Conventional introgression breeding is a well-established method for transferring targeted genes like *Xa21* but often leads to the co-introduction of undesirable donor alleles, necessitating multiple backcrosses and selfing generations to restore the genetic background of the elite recipient parent [22–24].

Marker-assisted selection (MAS) has significantly improved the precision and efficiency of breeding programs by enabling accurate tracking of target genes across generations [22]. MAS has been successfully employed to introgress *Xa21* into various susceptible cultivars, such as 'RD47' [25], 'CB 174 R' [22], 'Ciherang' [26], and 'PSL2' [6]. However, MAS has limitations in assessing genome-wide recovery of the recurrent parent, often relying on a limited number of markers that do not capture the full genetic composition.

By contrast, whole genome sequencing (WGS) provides high-resolution, genome-wide insights into gene introgression patterns and the genetic compositions of the breeding lines [27, 28]. Recent studies have demonstrated the utility of WGS in characterizing introgression lines, such as identifying *Heading-date 16* homozygous segregants in rice BC_5F_2 lines [29] and quantifying introgressed regions in common bean cultivars with over 92.65% accuracy [30]. Despite this potential, WGS has not been widely integrated into rice breeding programs to validate genetic background recovery in *Xa21*-based introgression lines.

This study applied WGS to select advanced backcross lines carrying the *Xa21* gene in the BC_5F_6 generation, followed by phenotypic confirmation in the BC_5F_7 generation. The introgression of *Xa21* into the Thai elite cultivar 'PSL2' was previously achieved through marker-assisted backcrossing [6]. 'PSL2' was chosen as the recurrent parent because of its high yield potential, superior grain quality, and resistance to major insect pests—traits that are essential for rice production in Thailand. WGS was integrated with MAS to identify lines that possess the *Xa21* gene and also exhibited high genetic similarity to the recurrent parent, ensuring improved BB resistance while maintaining desirable agronomic characteristics.

The remainder of this paper describes the rice materials and experimental procedures used to evaluate agronomic performance and BB resistance in the BC_5F_6 and BC_5F_7 generations along with WGS and genome composition analysis. The results section presents phenotypic evaluations, genetic compositions, and relationship analyses followed by genome distribution patterns of the introgression lines. The discussion elaborates on trait selection, the application of WGS, heterosis effects, and resistance performance, with the key findings and future perspectives for BB-resistant rice breeding presented as the conclusions.

2. Materials and Methods

2.1. Rice Materials

An overview of the experimental workflow is illustrated in Figure 1. The rice introgression lines 'PSL2-*Xa21*' at the BC_5F_6 generation were developed using MAS within a backcross breeding framework under greenhouse conditions at the Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Thailand, as described by Meesa et al. [6]. The F_1 hybrids were generated by crossing 'PSL2' (recipient parent) with 'IRBB21' (donor parent). The F_1 plants were then backcrossed to 'PSL2' for five generations (BC_5F_1) to recover the genetic background of the recipient parent. These BC_5F_1 plants were subsequently self-pollinated over five generations to obtain BC_5F_6 progenies with increased homozygosity. The presence of the *Xa21* locus was verified using a direct PCR assay with the MAS marker pTA248, following the method described by Meesa et al. [6].

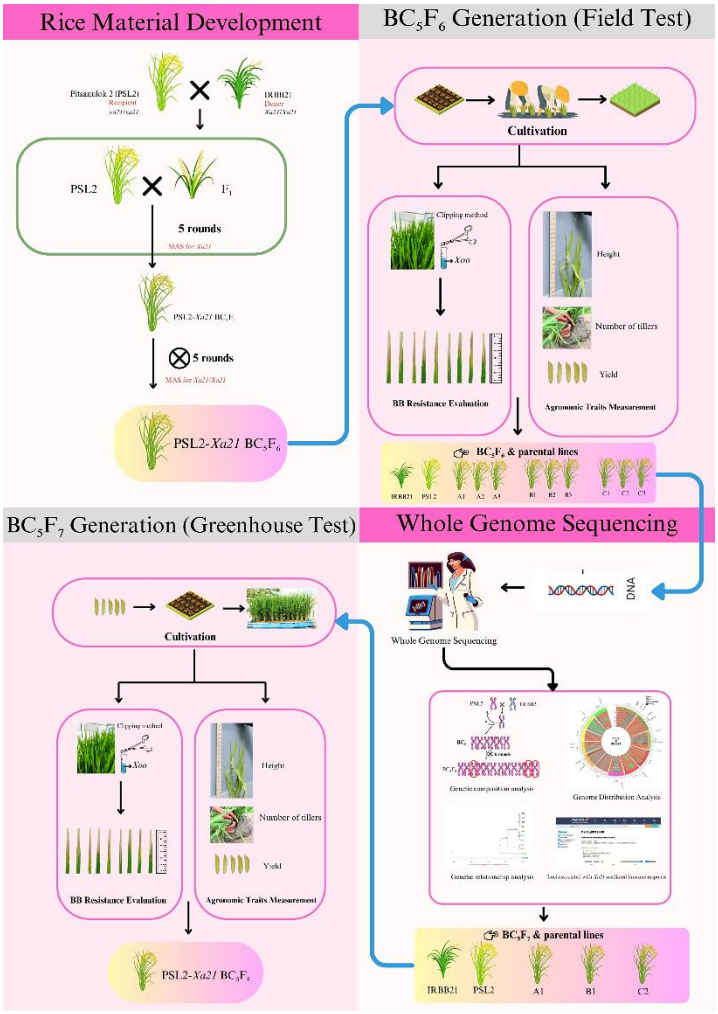

Figure 1. Schematic overview of the experimental workflow used to develop and evaluate the BC_5F_6 and BC_5F_7 'PSL2-*Xa21*' introgression lines

2.2. Agronomic Performance and Bacterial Blight Resistance Analysis

2.2.1. BC_5F_6 Generation

Three introgression lines and the parental lines were grown in a test cultivation field at the Rice Research Center, Phitsanulok Province, Thailand. The seeds were germinated in trays, with one seed per cell and 20 seeds per line. Seven-day-old seedlings were transplanted into the field, with five seedlings per row and four rows per line. Fertilizer was applied as 16-16-8 (15.6 g/m^2) at 7 days after planting and 46-0-0 (31.25 g/m^2) at 30 days after planting, with water maintained at a depth of 10 cm throughout the planting period.

Cultivation was conducted from October 2022 to February 2023 (wet to dry season transition), with an average daily temperature of 25.3 °C (range: 20.0–28.5 °C) and average daily humidity of 78.0% (range: 53.3–95.7%). At 60 days after germination (53 days after planting), the plants were inoculated with *Xoo* strain *Xoo*22PRRC [31], at a concentration of 2.5×10^8 cfu/mL, using the leaf-clipping method [32], BB resistance was evaluated 21 days post-inoculation based on the percentage of diseased leaf area following the Standard Evaluation System for Rice (field test) [33]. Resistance categories were defined as: resistant (R) = 1–5%, moderately resistant (MR) = 6–12%, moderately susceptible (MS) = 13–25%, susceptible (S) = 26–50%, and highly susceptible (HS) = 51–100%.

BB resistance levels were assessed using 10 plants per line. Agronomic traits, including plant height, number of tillers, 100-grain weight, and grain yield per hill, were measured at maturity using three plants for each parental line and five plants for each introgression line. Plant height was measured from the soil surface to the tip of the tallest panicle. The number of tillers was counted per plant. Grain yield per hill was calculated after harvesting, threshing, and drying, and the 100-grain weight was measured using a precision balance.

2.2.2. BC_5F_7 Generation

Nine selected introgression lines and the parental lines were grown under greenhouse conditions at the Faculty of Science, Naresuan University, Thailand. The seeds were germinated in trays (one seed per cell, 20 seeds per line).

Seven-day-old seedlings were transplanted into square plastic ponds (170 cm width × 100 cm length × 40 cm height) filled with 25 cm of soil sourced from rice fields. Each line was planted with five seedlings per row and four rows per line. The plants were grown under natural sunlight and ventilation, maintaining a water depth of 10 cm. Fertilizer application followed the same protocol as in the BC_5F_6 generation.

Cultivation was conducted from January to May 2024 (dry season), with an average daily temperature of 29.9 °C (range: 21.0–34.8 °C) and average daily humidity of 65.5% (range: 49.7–89.9%). Inoculation and resistance evaluation were performed as in the BC_5F_6 generation. For the greenhouse setting, resistance was assessed based on lesion length following greenhouse test criteria [33] as resistant (R) = 0–5 cm, moderately resistant (MR) ≥ 5–10 cm, moderately susceptible (MS) ≥ 10–15 cm, susceptible (S) ≥ 15–20 cm, and highly susceptible (HS) ≥ 20 cm.

BB resistance levels were evaluated using five plants per line. Agronomic traits were measured from five plants at maturity using the same criteria described for the BC_5F_6 generation.

2.3. Whole Genome Sequencing

Genomic DNA was extracted from the fresh leaves of nine selected BC_5F_6 introgression lines (three plants per line) and the parental lines using the PureDireX Genomic DNA Isolation Kit (Plant) (Bio-helix, Taiwan) following the manufacturer's protocol, with an extended lysis step of 1 hour. DNA quality and quantity were assessed using a NanoDrop™ Lite Spectrophotometer (Thermo Scientific, USA), and DNA integrity was verified by agarose gel electrophoresis (1% agarose, TAE buffer, 100 V, 30 minutes). The qualified samples were sent to Ward Medic Co., Ltd. for quality control (QC) analysis.

For library preparation, 0.2 μg of QC-approved DNA was fragmented to ~350 bp by sonication. The DNA fragments were subjected to end polishing, A-tailing, and ligation with full-length Illumina adapters. Size selection and PCR amplification were followed by purification with the AMPure XP system. Library quality was verified using the Agilent 5400 system, and concentration (1.5 nM) was confirmed by real-time PCR. QC-pass libraries were pooled and sequenced using Illumina NovaSeq X Plus platforms with paired-end 150-bp (PE150) reads.

Bioinformatic processing began with the conversion of raw sequencing data into short reads (FASTQ format). The reads were filtered to remove those with over 10 bases of adapter contamination, more than 10% ambiguous bases, or more than 50% of bases with a Phred score below 5.

High-quality reads were aligned to the IRGSP-1.0 reference genome (The International Rice Genome Sequencing Project) using the Burrows-Wheeler Aligner (BWA) with the parameters *mem -t 4 -k 32 -M*. Duplicate reads were subsequently removed using SAMtools and Picard. Variant calling for SNPs and Indels was performed with SAMtools using the options *-C 50 -mpileup -m2 -F 0.002 -d 1000*. Variants were filtered based on a minimum read depth of 4 and a mapping quality score greater than 20. Functional annotation of the variants was carried out using ANNOVAR, incorporating gene and region annotations from UCSC-known genes.

2.4. Genetic Relationships and Genome Distribution Analysis

The high-quality reads for each introgression line were converted into ABH format using TASSEL version 5.2.87 [34]. At each map position, alleles from the recurrent parent ('PSL2'), donor parent ('IRBB21'), and heterozygous loci were denoted as A, B, and H, respectively. Genetic similarity was calculated as the proportion of alleles matching either parent relative to the total number of alleles.

The genome-wide genotype distribution across chromosomes for each introgression line was visualized using Circos software [35]. Putative genes associated with *Xa21*-mediated BB resistance were identified using a BLAST search against the Rice Annotation Project database (*https://rapdb.dna.affrc.go.jp/*).

Genetic relationships among the parental lines and nine introgression lines were analyzed using the unweighted pair group method with arithmetic averaging (UPGMA) in MEGA11 [36], with genetic distances calculated using the Maximum Composite Likelihood method with 1,000 bootstrap replicates to ensure robustness.

2.5. Data Analysis

The experiment used a completely randomized design (CRD) with three to five biological replicates. Results were expressed as mean ± standard deviation. A dot plot for BB-lesion length in the BC_5F_7 generation was generated using the PlotsOfData web application (*https://huygens.science.uva.nl/PlotsOfData/*) [37]. Statistically significant differences between the introgression lines and parental lines in phenotypic data—including agronomic traits and BB-lesion lengths in the BC_5F_6 and BC_5F_7 generations—were determined using one-way analysis of variance (ANOVA), followed by Duncan's new multiple range test (DMRT) at a significance level of $p \leq 0.05$.

3. Results

3.1. Agronomic Performance and BB Resistance of 'PSL2-*Xa21*' Introgression Lines in The BC_5F_6 Generation

The morpho-agronomic traits and BB resistance of three introgression lines in the BC_5F_6 generation were evaluated under natural field conditions to assess the impact of the *Xa21* gene introgression performance. All three lines exhibited significantly greater plant height than the parental cultivars, ranging from 113.6 ± 3.4 cm to 118.0 ± 2.0 cm, compared to 93.7 ± 2.1 cm in the recipient parent 'PSL2' and 98.0 ± 6.2 cm in the donor 'IRBB21'. Grain yield also improved in the introgression lines, particularly in line 'B' (69.90 ± 22.86 g/hill), indicating enhanced productivity potentially due to favorable gene combinations. The 100-grain weight of the introgression lines was slightly lower than 'PSL2' but higher than the donor parent 'IRBB21'. The tiller number did not differ significantly among the introgression lines and the parents (Table 1). These results suggested that the introgression lines showed potential to contribute to increased yield.

BB resistance assessment after inoculation with *Xoo*16PK002 revealed that lines 'A' and 'B' exhibited moderate resistance (MR), while line 'C' showed full resistance (R), similar to the donor parent 'IRBB21'. These findings confirmed the successful introgression of *Xa21* and its functional expression in line 'C', while the partial resistance in lines 'A' and 'B' indicated variations in gene expression or additional modifiers in the genetic background.

These results demonstrated that the BC_5F_6 'PSL2-*Xa21*' introgression lines simultaneously improved agronomic traits and BB disease resistance. The performances of lines 'B' and 'C' showed promise due to their high yield and BB resistance. These traits are valuable for breeding programs aiming to enhance productivity and disease resilience. To further explore their genetic basis, the three top-performance plants in each line were selected for WGS analysis.

Table 1. Field morphological and agronomic characteristics of the BC_5F_6 'PSL2-*Xa21*' introgression lines compared with the donor parent 'IRBB21' and the recurrent parent 'PSL2'

Cultivar/Line	Height (cm)	No. of tillers	100-grain weight (g)	Grain yield (g/hill)	BB resistant level [a]
IRBB21 (donor)	98.0 ± 6.2 b	12.7 ± 5.5 b	2.32 ± 0.22 c	34.25 ± 22.17 b	R
PSL2 (recipient)	93.7 ± 2.1 b	26.0 ± 11.4 a	3.07 ± 0.03 a	22.35 ± 0.60 b	S
A	118.0 ± 2.0 a	17.4 ± 2.3 ab	2.63 ± 0.09 b	51.36 ± 2.65 ab	MR
B	116.6 ± 2.4 a	17.4 ± 1.8 ab	2.77 ± 0.14 b	69.90 ± 22.86 a	MR
C	113.6 ± 3.4 a	17.4 ± 2.3 ab	2.64 ± 0.04 b	55.25 ± 9.95 ab	R

Note: Rice was grown in natural field conditions. Data are mean ± SD of three plants for parental lines and -five plants for introgression lines. Different letters in the same column indicate statistically significant differences according to DMRT at $p \leq 0.05$.

[a] Bacterial blight (BB) resistant levels: R, resistant; MR, moderately resistant; S, susceptible.

3.2. Genetic Composition Analysis

WGS was employed to analyze the genetic compositions of nine 'PSL2-*Xa21*' introgression lines in the BC_5F_6 generation. The number of DNA segment reads ranged from 105,298 to 115,606, while the average segment length varied between 2,967.95 bp and 3,288.41 bp. High genome coverage was achieved in all lines, with coverage lengths ranging from 343.11 Mb to 346.26 Mb, corresponding to 91.93% to 92.77% of rice reference genome—indicating high-quality sequencing data and comprehensive representation of the genetic content (Table 2).

Seven of the nine introgression lines (excluding 'B1' and 'B2') exhibited high genetic similarity to the recurrent parent ('PSL2'), ranging from 71.83% to 73.31%, and low similarity to the donor parent ('IRBB21'), ranging from 18.00% to 19.44%. By contrast,'B1' and 'B2' retained a high proportion of the donor genome (23.22 and 23.21, respectively) and lower proportion of the recurrent genome (68.35% and 68.25%, respectively) (Table 2).

These findings highlighted the effectiveness of backcross selection in progressively recovering the recurrent parent genome. Five rounds of backcross generation followed by six generations of self-pollination (BC_5F_6) contributed to increased homozygosity throughout the genome. For further genetic stabilization, the line with the highest homozygosity in each group ('A1', 'B1', and 'C2') was selected and self-pollinated to develop the 'BC_5F_7' generation and used in subsequent experiments to develop desirable traits.

Table 2. Whole genome resequencing summary and percentage of homozygous genotypes in BC_5F_6 'PSL2-*Xa21*' introgression lines derived from the donor ('IRBB21') and recurrent ('PSL2') parental lines

Line	Number of reads [a]	Average length (bp/segment)	Coverage length [b]		Genetic similarity (%) to	
			(Mb)	(%)	IRBB21	PSL2
A1	105,298	3,288.41	346.26	92.77	19.40	72.17
A2	114,070	3,015.80	344.01	92.17	19.44	71.83
A3	112,433	3,053.30	343.29	91.97	19.36	71.88
B1	112,446	3,059.38	344.01	92.17	23.22	68.35
B2	112,402	3,063.88	344.39	92.27	23.21	68.25
B3	114,598	2,999.99	343.79	92.11	18.05	73.31
C1	115,606	2,967.95	343.11	91.93	18.11	73.19
C2	114,589	2,998.50	343.59	92.06	18.19	73.14
C3	113,936	3,018.85	343.96	92.15	18.00	73.12

[a] Number of segments: Represents the total DNA segments mapped across the 12 rice chromosomes.

[b] Coverage length (%): Indicates the proportion of sequenced reads relative to the complete rice genome.

3.3. Genetic Relationship Analysis

The genetic relationships among the parental lines ('PSL2' and 'IRBB21') and the nine BC_5F_6 'PSL2-*Xa21*' introgression lines (listed in Table 2) were examined using a UPGMA dendrogram based on WGS reads (Figure 2). The analysis revealed two main clusters. Cluster I comprised the donor parent 'IRBB21', which exhibited the greatest genetic distance (~0.4). By contrast, Cluster II included the recurrent parent 'PSL2' and all nine introgression lines reflecting a closer genetic relationship, with a genetic distance of approximately 0.28.

Cluster II was further divided into two sub-clusters. Sub-cluster II-i contained seven introgression lines ('A1'–'A3', 'B3', and 'C1'–'C3') which exhibited the highest genetic similarity to 'PSL2', with the smallest genetic distance (~0.048). Sub-cluster II-ii consisted of two introgression lines ('B1' and 'B2') which showed slightly higher genetic distance values (~0.05) but were closely related to the recurrent parent.

These findings concurred with the results of the genetic composition analysis (section 3.2), confirming that all the introgression lines shared a high degree of genomic similarity with 'PSL2', supporting the conclusion that the BC_5F_6 'PSL2-*Xa21*' lines recovered the genetic background of the recurrent parent while successfully retaining the target resistance gene *Xa21*.

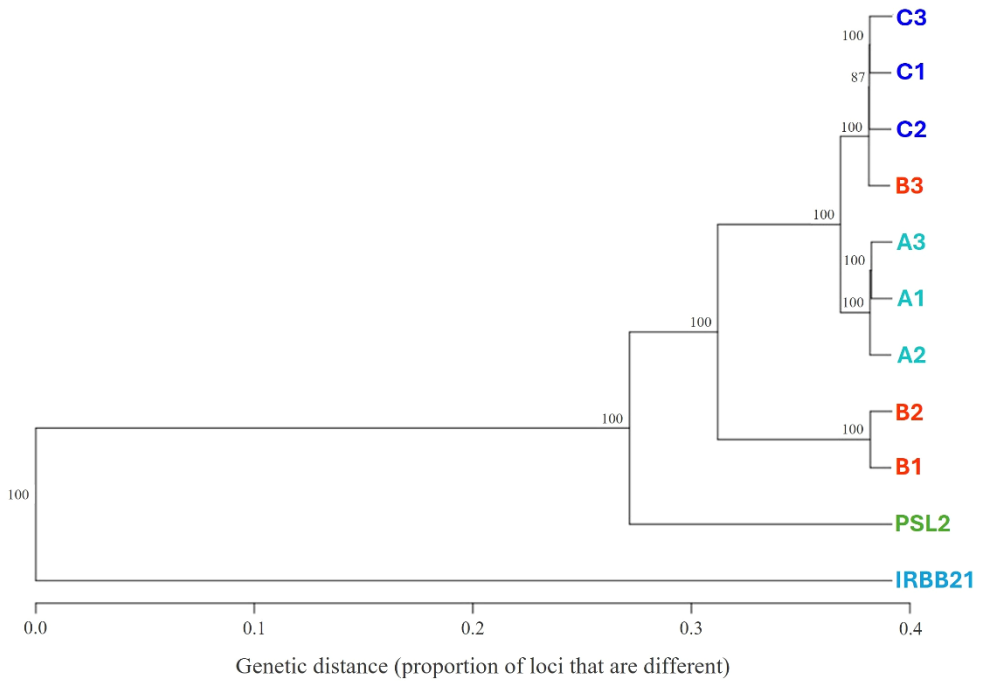

Figure 2. Dendrogram showing the genetic relationships among the donor parent ('IRBB21'), the recipient parent ('PSL2'), and the BC_5F_6 'PSL2-*Xa21*' introgression lines ('A1'–A3, 'B1'–'B3', 'C1'–'C3'). The tree was constructed using the UPGMA method in MEGA11. Bootstrap values (1-100) at the nodes indicate the confidence levels of the groupings.

3.4. Genome Distribution of Introgression Lines

WGS data from nine BC_5F_6 introgression lines were used to generate Circos plots, visualizing the genome-wide distribution of homologous DNA segments relative to the donor parent ('IRBB21') and the recurrent parent ('PSL2') across all 12 rice chromosomes (Figure 3). These circular heatmaps provided a high-resolution view of the genomic composition in each line for a deeper understanding of introgression patterns.

The Circos plots revealed that most of the chromosomal regions in all nine lines were highly homologous to 'PSL2', particularly chromosomes 1 to 5, 10, and 12, consistent with the advanced backcrossing approach used in developing these lines that retained the genetic background of the recurrent parent while incorporating specific traits from the donor. By contrast, chromosome 11 consistently displayed a greater proportion of 'IRBB21'-derived segments, reflecting the targeted introgression of the BB resistance gene *Xa21* located on this chromosome. This pattern confirmed the successful introgression of the *Xa21*-containing segment while minimizing linkage drag elsewhere in the genome.

Heterozygous segments—regions containing alleles from both 'IRBB21' and 'PSL2'—were predominantly scattered across chromosomes 3, 6, 7, 8, and 9. These heterozygous zones may represent recombination hotspots or regions under selection for complex traits such as yield or stress tolerance, where favorable alleles from both parents may be retained. The relatively low levels of heterozygosity on the other chromosomes indicated a high degree of genome stabilization in later backcross generations.

A comparative analysis of the Circos plots also supported the clustering patterns observed in the dendrogram (Figure 2). The introgression lines 'B1' and 'B2', grouped into sub-cluster II-ii, exhibited distinct genomic profiles compared to the other lines (sub-cluster II-i), including a higher proportion of 'PSL2' DNA on chromosome 5 and increased 'IRBB21' segments on chromosome 12 (Figure 3). These variations suggested subtle phenotypic differences between these sub-clusters, while within advanced backcross lines, residual variations contributed to genetic diversity.

Functional annotation of the WGS reads identified 17 candidate genes associated with *Xa21*-mediated immunity to BB (Table 3), grouped into five functional categories as:

1) Receptor kinase-like protein: The gene *Os11g0559200* (*Xa21*) is a well-known immune receptor located on chromosome 11, which plays a pivotal role in pathogen recognition and activation of downstream immune responses.

2) Binding protein in the *Xa21* signaling pathway: Six genes including *Os01g0771200* (*XB24*), *Os03g0821300* (*XB15), Os05g0112000 (XB13), Os09g0513000 (XB25)*, and *Os09g0417800 (XB10 or WRKY62)* modulate pattern-triggered immunity (PTI) and facilitate the transition to effector-triggered immunity (ETI), reflecting a multilayered defense strategy.

3) Regulators of *Xa21*-mediated immunity: Five genes—*Os01g0931400* (*ROX1*), *Os02g0320100* (*ROX2*), *Os06g0231300* (*ROX3*), *Os08g0278900* (*OsSDF2-1*), and *Os08g0440500* (*OsSDF2-2*)—are involved in nuclear signaling and endoplasmic reticulum quality control (ER-QC), ensuring proper folding and trafficking of defense-related proteins.

4) Leucine-rich repeat (LRR) domain proteins: *Os01g0809300* (*LRR1*) and *Os02g0553000* (*XIK1*) encoded pattern-recognition receptors that are essential for detecting pathogen-associated molecular patterns (PAMPs), reinforcing early immune responses.

5) *Xa21*-associated genes: *Os02g0115900* (*BiP*), *Os03g0170400* (*RLCK102*), and *Os03g0586700* (*PALD*) contribute to receptor stability, signal transduction, and post-translational regulation of immunity, highlighting the complexity of resistance mechanisms.

These findings confirmed the introgression of *Xa21* and associated genomic regions from 'IRBB21' into the 'PSL2' background and provided molecular evidence of the retention of key resistance genes. The diversity of immune-related functions encoded by these genes suggested a robust and multilayered resistance mechanism in the improved lines, which may confer durable protection against bacterial blight.

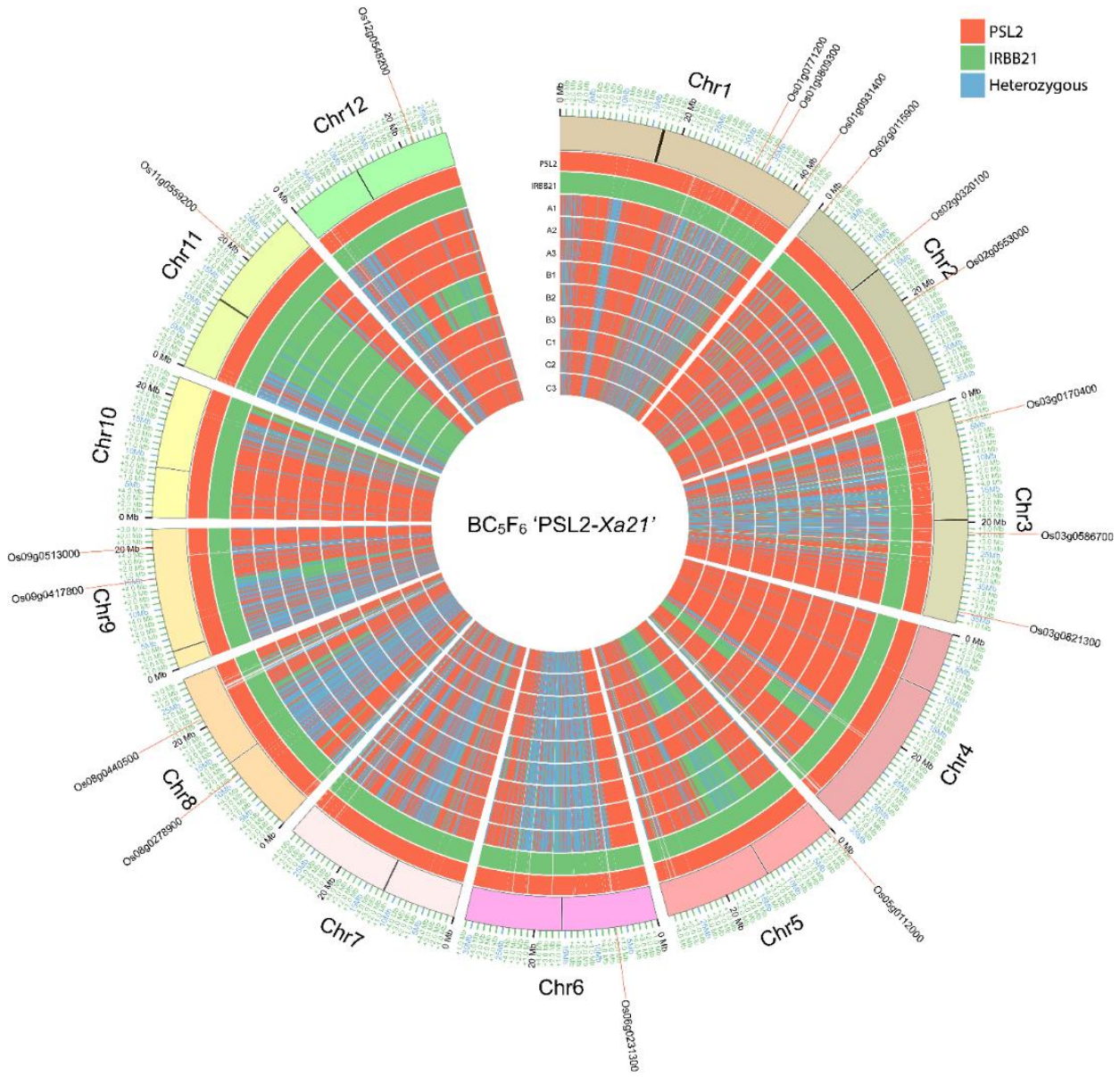

Figure 3. Genome-wide distribution of homologous segments from 'PSL2' (recurrent parent), 'IRBB21' (donor parent), and heterozygous regions across 12 rice chromosomes in nine BC_5F_6 'PSL2-*Xa21*' introgression lines. Circos plots were constructed using whole genome sequencing data to visualize the genomic composition of each line. Colored segments represent genomic regions derived from 'PSL2' (orange), 'IRBB21' (green), heterozygous combinations (blue), and non-covered regions (white). The outer ring indicates chromosomal positions. Loci associated with BB resistance are annotated with locus IDs (see Table 3 for details).

Table 3. Annotated gene loci associated with *Xa21*-mediated immune responses, predicted from WGS of BC_5F_6 'PSL2-*Xa21*' introgression lines

Chromosome: position [a]	Locus ID	Gene symbol	Description
chr01: 32543304..32545191 (+ strand)	*Os01g0771200*	*XB24*	*Xa21* binding protein 24, ATPase, Inhibition of pattern recognition receptor (PRR) -mediated immunity (*Os01t0771200-01*)
chr01: 34379096..34383084 (+ strand)	*Os01g0809300*	*LRR1, OsLRR1*	Extracellular leucine-rich repeat (eLRR) domain protein, *XB21* interacting protein, *Xa21*-mediated immune response, Regulation of plant responses against pathogen (*Os01t0809300-01*)
chr01: 40868709..40871916 (- strand)	*Os01g0931400*	*ROX1*	Similar to thiamin pyrophosphokinase 1. (*Os01t0931400-01*); Thiamine pyrophosphokinase, Positive regulation of XA21-mediated immunity, Defense response (*Os01t0931400-02*)
chr02: 838743..842672 (- strand)	*Os02g0115900*	*BiP1, BiP3*	ER-localized chaperone HSP70, ER quality control for seed storage proteins during seed maturation, Regulation of *Xa21* protein stability and processing, Resistance to *Xoo*, (*Os02t0115900-01*); Endosperm lumenal binding protein. (*Os02t0115900-02*); Similar to Endosperm lumenal binding protein. (*Os02t0115900-03*)
chr02: 12758249..12762205 (- strand)	*Os02g0320100*	*ROX2*	Bacterial Fmu (Sun)/eukaryotic nucleolar NOL1/Nop2p domain containing protein. (*Os02t0320100-01*); A member of the NOL1/NOL2/sun gene family, Positive regulation of XA21-mediated immunity (*Os02t0320100-02*)

chr02: 20857044..20861343 (- strand)	*Os02g0553000*	*XIK1*	Protein kinase, a catalytic domain containing protein. (Os02t0553000-01); Leucine-rich repeat receptor-like kinase (LRR-RLK), Regulation of Xa21-mediated disease resistance (*Os02t0553000-02*)
chr03: 3767426..3771538 (- strand)	*Os03g0170400*	*RLCK102*	Similar to serine/threonine-protein kinase NAK. (*Os03t0170400-01*); Receptor-like cytoplasmic kinase, Xa21-mediated disease resistance to Xoo, Negative regulation of rice development through BR signaling (*Os03t0170400-02*)
chr03: 21647446..21659950 (- strand)	*Os03g0586700*	*PALD*	Paladin, Tyrosine phosphatase-like protein, XA21-mediated immunity (*Os03t0586700-01*)
chr03: 34474697..34478370 (- strand)	*Os03g0821300*	*XB15*	Protein phosphatase 2C, Negative regulation of XA21-mediated innate immune response and cell death (*Os03t0821300-01*); Similar to Protein phosphatase 2C 35. (*Os03t0821300-02*)
chr05: 640321..643316 (- strand)	*Os05g0112000*	*XB3*	E3 ubiquitin ligase, XA21-mediated disease resistance (*Os05t0112000-01*); Similar to Receptor-like kinase Xa21-binding protein 3. (*Os05t0112000-04*); Similar to Receptor-like kinase Xa21-binding protein 3. (*Os05t0112000-05*)
chr06: 6794151..6796570 (+ strand)	*Os06g0231300*	*ROX3*	Nuclear migration protein, Negative regulation of XA21-mediated immunity (*Os06t0231300-01*)
chr08: 10810395..10813333 (- strand)	*Os08g0278900*	*OsSDF2-1*	Stromal cell-derived factor 2, Endoplasmic reticulum-quality control (ER-QC) protein, XA21-mediated immunity (*Os08t0278900-01*)
chr08: 21433598..21436735 (- strand)	*Os08g0440500*	*OsSDF2-2*	Stromal cell-derived factor 2, Endoplasmic reticulum-quality control (ER-QC) protein, XA21-mediated immunity (*Os08t0440500-01*)
chr09: 14992918..14994888 (- strand)	*Os09g0417800*	*XB10, WRKY62*	WRKY transcription factor, Transcriptional repressor, Pathogen defense (*Os09t0417800-01*); Splicing variant of OsWRKY62, Pathogen defense (*Os09t0417800-02*)
chr09: 19961104..19964106 (+ strand)	*Os09g0513000*	*XB25, OsBIANK1*	XA21 binding protein 25, Plant-specific ankyrinrepeat (PANK) family protein, XA21-mediated disease resistance (*Os09t0513000-01*)
chr11: 20802978..20806262 (+ strand)	*Os11g0559200*	*Xa21*	Receptor kinase-like protein, Bacterial leaf blight resistance, Disease resistance (*Os11t0569733-01*)
chr12: 22173286..22180480 (- strand)	*Os12g0548200*	*XB21*	Hypothetical conserved gene. (Os12t0548200-01); Auxilin-like protein, Type III J-protein, Positive regulation of resistance to *Xoo*, Regulation of cell death (*Os12t0548200-02*)

[a] The plus symbol (+) indicates transcription in the left-to-right orientation, while the minus symbol (-) indicates transcription in the right-to-left orientation. Gene information was obtained from the Rice Annotation Project database.

3.5. Agronomic Performance and BB Resistance of 'PSL2-*Xa21*' Introgression Lines in The BC_5F_7 Generation

To further evaluate agronomic traits and BB resistance, three BC_5F_6 introgression lines ('A1', 'B1', and 'C2') exhibiting the highest homozygosity and genetic similarity to the recurrent parent 'PSL2' were advanced to the BC_5F_7 generation through self-pollination. Individual seeds were collected from separate parents, resulting in nine BC_5F_7 introgression lines ('A1-1', 'A1-2', 'A1-3', 'B1-1', 'B1-2', 'B1-3', 'C2-1', 'C2-2', and 'C2-3'). These were then evaluated for both agronomic performance and BB resistance under greenhouse conditions.

As shown in Figure 4, all BC_5F_7 lines exhibited whole-plant morphology comparable to or better than 'PSL2' at 60 days after germination, with. quantitative data in Table 4 further confirming this trend. Particularly, 'B1-3' demonstrated significantly improved traits, including plant height (108.0 cm), number of tillers (15.4), and grain yield (32.4 g/hill) while 'C2-3' exhibited improved height (107.4 cm) and number of tillers (16.4), with grain yield (21.45 g/hill) similar to 'PSL2'. These findings suggested the successful recovery of elite agronomic traits in the advanced backcrossed lines.

Resistance to BB, assessed by lesion lengths following *Xoo* inoculation, revealed variations among the BC_5F_7 lines (Figure 5). The resistant donor parent 'IRBB21' showed minimal lesion development (4.4 ± 1.3 cm), whereas the susceptible recurrent parent 'PSL2' exhibited extensive lesioning (37.1 ± 2.8 cm). Six introgression lines ('A1-2', 'A1-3', 'B1-2', 'B1-3', 'C2-2' and 'C2-3') displayed significantly shorter lesion lengths than 'PSL2' and were classified as moderately resistant (MR). The shortest lesions among these were recorded in 'A1-2' (7.4 ± 1.0 cm), 'A1-3' (7.9 ± 1.0 cm), 'B1-2' (6.8 ± 0.9 cm), and 'C2-2' (6.9 ± 1.2 cm), suggesting effective partial expression of *Xa21*-mediated resistance.

Despite the shared origin of *Xa21* introgression, three BC_5F_7 lines ('A1-1', 'B1-1' and 'C2-1') were moderately susceptible (MS), indicating possible variability in gene expression, recombination near the resistance locus, or background genome interactions affecting resistance. These phenotypic differences are shown in the upper panel of Figure 5, with lesion development on the leaves of the MR lines appears visibly less severe than in the MS lines or the susceptible parent.

None of the BC_5F_7 introgression lines reached the full resistance level of 'IRBB21' but the dual performance of 'B1-3' and 'C2-3'—showing superior agronomic traits and moderate BB resistance—highlighted their promise as breeding candidates. These lines warrant prioritization for further advancement to the BC_5F_8 generation and in-depth field evaluations to validate their potential for BB resistance and yield improvement under diverse conditions.

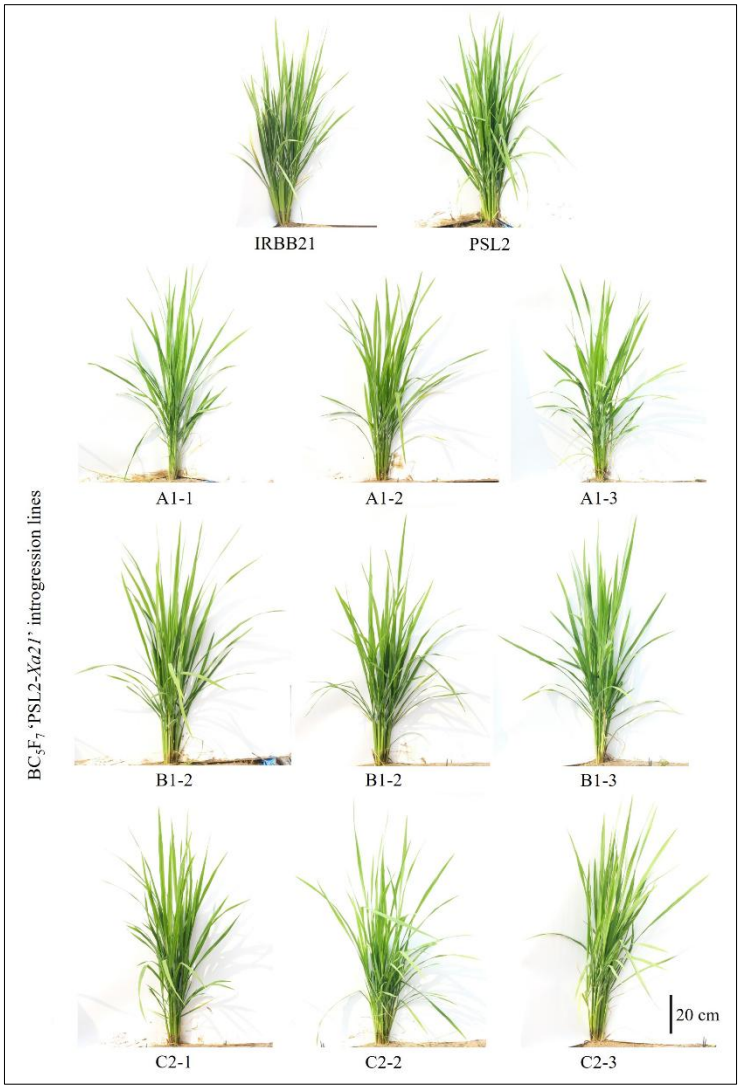

Figure 4. Whole-plant morphology of the donor parent ('IRBB21'), the recipient parent ('PSL2'), and the BC_5F_7 'PSL2-*Xa21*' introgression lines grown under greenhouse conditions with natural sunlight and ventilation. The photographs were taken 60 days after germination.

Table 4. Greenhouse morphology and agronomic characteristics of the BC_5F_7 'PSL2-*Xa21*' introgression lines compared with the donor parent 'IRBB21' and the recurrent parent 'PSL2'

Cultivar/Line	Height (cm)	No. of tillers	100-grain weight (g)	Grain yield (g/hill)	BB resistant level [a]
IRBB21 (donor)	84.4 ± 0.9 d	5.0 ± 1.0 e	2.22 ± 0.03 d	16.28 ± 0.47 c	R
PSL2 (recipient)	96.2 ± 5.5 c	8.0 ± 3.3 cde	3.05 ± 0.05 a	22.35 ± 0.60 b	S
A1-1	104.8 ± 3.9 ab	11.0 ± 3.1 a-d	2.65 ± 0.08 b	17.36 ± 0.94 c	MS
A1-2	101.0 ± 1.6 bc	12.6 ± 5.4 abc	2.32 ± 0.03 cd	10.05 ± 1.55 cd	MR
A1-3	106.4 ± 3.9 a	10.8 ± 4.9 bcd	2.66 ± 0.11 b	17.40 ± 3.11 c	MR
B1-1	99.6 ± 3.8 bc	12.0 ± 4.0 abc	2.70 ± 0.06 b	12.04 ± 0.56 c	MS
B1-2	96.3 ± 4.1 c	6.2 ± 1.6 de	2.33 ± 0.06 c	10.39 ± 1.96 cd	MR
B1-3	108.0 ± 6.2 a	15.4 ± 2.5 ab	2.62 ± 0.07 b	32.41 ± 2.20 a	MR
C2-1	98.6 ± 2.7 c	8.2 ± 3.6 cde	2.69 ± 0.04 b	7.58 ± 0.11 d	MS
C2-2	99.3 ± 4.8 bc	8.4 ± 2.9 cde	2.36 ± 0.02 c	11.79 ± 1.28 c	MR
C2-3	107.4 ± 3.8 a	16.4 ± 6.3 a	2.62 ± 0.09 b	21.45 ± 2.63 b	MR

Note: Rice was grown in a greenhouse with natural ventilation and sunlight. Data are mean ± SD of 5 plants. Different letters within the same column indicate statistically significant differences according to DMRT at $p \leq 0.05$.

[a] Bacterial blight (BB) resistant levels: R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.

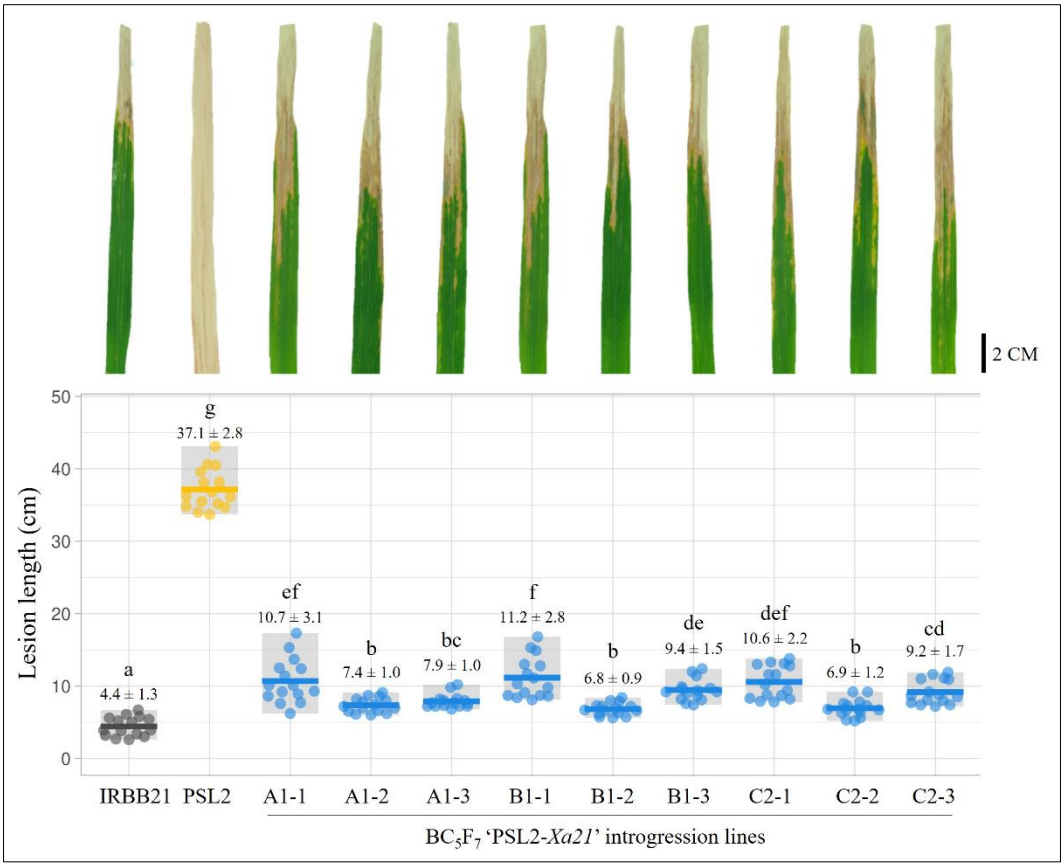

Figure 5. Bacterial blight (BB) symptoms (upper panel) and lesion lengths (lower panel) observed at 21 days after inoculation with *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) in BC_5F_7 'PSL2-*Xa21*' introgression lines, compared with the donor parent ('IRBB21') and the recipient parent ('PSL2'), grown under greenhouse conditions with natural sunlight and ventilation. In the lower panel, each dot represents the lesion length from a single leaf (2 leaves per plant, 5 plants per line). The horizontal line indicates the mean, and the gray box represents the data range. Values above each bar denote mean ± SD. Different letters above bars indicate statistically significant differences among means, determined by DMRT at $p \leq 0.05$.

4. Discussion

4.1. Agronomic Trait Selection in Introgression Lines

Field evaluations revealed that three BC_5F_6 'PSL2-*Xa21*' introgression lines exhibited significant improvements in plant height and grain yield relative to the recurrent parent 'PSL2' and the donor parent 'IRBB21'. The number of tillers did not significantly differ among genotypes, but the enhancement in key agronomic traits suggested that recombination between the donor and the recurrent parent genomes facilitated the expression of favorable alleles. This phenomenon concurred with the concept of transgressive segregation, where novel allele combinations arising from recombination result in superior trait performance [38].

These findings aligned with the objective of backcross breeding, retaining desirable traits from the recurrent parent while introducing novel resistance genes from the donor parent [6, 26]. Similarly, agronomic improvements have been reported in previous studies incorporating *Xa21,* such as in 'CB 174 R' [22], 'RD47' [25], and 'Ciherang' [26]; however, those studies often prioritized resistance introgression without a detailed assessment of recurrent genome recovery or agronomic enhancement. By contrast, our study demonstrated significant phenotypic improvements and substantial genomic recovery.

The BC_5F_6 lines also displayed enhanced BB resistance, an essential trait for sustaining rice productivity under pathogen pressure. Lines 'A' and 'B' showed moderate resistance (MR), while line 'C' displayed full resistance (R), comparable to the donor 'IRBB21'. This underscored the effective introgression and expression of *Xa21*, a well-characterized gene that activates strong defense responses against *Xoo*. Zhang et al. [39] observed elevated *Xa21* expression in the 'Kitaake' background conferring resistance to *Xoo* strain PXO99 without yield penalties under greenhouse conditions.

This study combined genetic recovery data, field-based performance, and detailed phenotypic evaluation as an integrated assessment that provided more comprehensive evidence of the effectiveness of the backcross breeding strategy, highlighting the successful transfer of BB resistance and also the recovery of high genetic similarity to the recurrent parent along with agronomic gains.

4.2. WGS Analysis Among Introgression Lines

WGS analysis of the BC_5F_6 'PSL2-*Xa21*' introgression lines confirmed the high genetic recovery of the recurrent parent genome. Among the nine lines examined, seven ('A1', 'A2', 'A3', 'B3', 'C1', 'C2', and 'C3') exhibited high DNA sequence homology to 'PSL2', ranging from 71.83% to 73.31% (Table 2). This supported the effectiveness of repeated backcrossing and phenotypic selection in restoring the recurrent parent genome while introducing the *Xa21* gene from the donor 'IRBB21'. These lines also displayed predominantly homogeneous loci, indicating that the backcrossing strategy was effective in recovering the genetic composition of 'PSL2'. Theoretical models of introgression suggested that repeated backcrossing, coupled with selection for the recipient parent traits, progressively increased the proportion of the recipient parent genomes in subsequent generations [22, 40]. This study confirmed backcross breeding as a reliable approach for retaining key traits of the recurrent parent while incorporating specific desirable traits from the donor parent.

By contrast, lines 'B1' and 'B2' showed noticeably lower similarity to 'PSL2' and harbored a greater proportion of 'IRBB21' loci (Table 2). These deviations suggested a higher retention of donor parent genomic segments, likely due to random segregation events or selective advantage conferred by certain donor-derived loci [41]. These lines may have preserved donor alleles influencing traits other than BB resistance, such as stress tolerance or secondary metabolite production [42], highlighting the importance of scrutinizing such deviations to refine future backcrossing and selection strategies, particularly in managing regions of heterozygosity.

The clustering pattern observed in the UPGMA dendrogram (Figure 2) reinforced these genetic distinctions. Most introgression lines were grouped in cluster II along with the recurrent parent 'PSL2', illustrating their high genomic resemblance, while 'B1' and 'B2' formed a distinct sub-cluster within cluster II, indicating relatively divergent genomic composition. This clustering pattern complemented the similarity index data from Table 2 and supported the genomic heterogeneity revealed by sequencing.

Further insight into chromosomal contributions was provided by the Circos plot analysis (Figure 3), which visualized introgression patterns at the chromosomal level. Homologous regions to 'PSL2' were dominant on chromosomes 1–5, 10, and 12, indicating the successful recovery of large genomic segments from the recurrent parent. Conversely, chromosome 11 contained a notable proportion of donor-derived sequences, suggesting that donor loci on this chromosome were either linked to *Xa21* or retained due to genetic hitchhiking or pleiotropic effects [43, 44]. Heterozygous regions were largely distributed on chromosomes 3, 6, 7, 8, and 9, representing potential hotspots for genomic mixing and novel allelic interactions. The presence of heterozygosity in specific genomic regions may have functional implications. Gene interactions within these heterozygous loci may influence trait expressions such as plant height or stress response. Such epistatic interactions contribute heterosis-like effects, even in later backcross generations. Examining these regions in greater detail may uncover novel genetic combinations beneficial for breeding.

WGS was proved to be a powerful tool in introgression breeding by enabling detailed genome-wide assessment of donor-recipient recombination [45]. Previous studies have leveraged WGS for similar purposes—for instance, identifying homozygous introgression lines carrying the *Heading-date 16* gene in BC_5F_2 rice [29] or recovering high genomic similarity (92.65%) to the recurrent parent in common bean [30]. This study built on these approaches by demonstrating the feasibility of combining high genomic recovery with disease resistance in BC_5F_6 rice populations.

Integration with complementary and phenotypic tools is recommended to strengthen the genomic inferences from WGS, while quantitative trait loci (QTL) mapping could link the observed agronomic traits with specific genomic regions, especially in heterozygous loci. Transcriptome (RNA-Seq) or proteome profiling could also validate the functional expression of *Xa21* and related defense pathways. Functional genomics approaches like CRISPR/Cas9 could further establish the causality of candidate genes, while multi-environmental field trials could clarify the stability of trait expression under diverse conditions. These approaches would provide a holistic understanding of genotype-to-phenotype relationships in introgression lines, strengthening the application of molecular breeding strategies.

4.3. Heterosis Effect in Introgression Lines

The backcross breeding approach effectively recovered the genome of the recurrent parent 'PSL2', while successfully introgressing the *Xa21* gene from the donor parent 'IRBB21'. The resulting BC_5F_7 lines exhibited hybrid vigor (heterosis), with significantly enhanced agronomic traits—including increased plant height, tiller number, and grain yield—compared to both parental lines. This enhanced performance was attributed to the combined action of QTL from both parents, particularly those associated with heterosis. These loci likely contributed to trait improvement through the dominance effect model, where the dominant alleles from each parent mask deleterious recessive alleles, leading to superior phenotypes in the hybrid progeny [46–48].

Synergistic interactions among the dominance alleles in the model enhanced trait expression. For example, in sorghum, the pyramided line 'i5' carrying five dominant QTL alleles (*qCL-1, qCL-6, qCL-7a, qCL-7b*, and *qCL-9*)

showed an 82.8 to 85.6% increase in culm length, significantly boosting biomass compared to the recurrent parent 'Tentaka' [49]. Similarly, in rice, heterozygous genotypes with *qSS7* and *qHD8* alleles were associated with the heterosis observed in the high-yielding hybrid variety 'Liangyoupei9' [50].

Functional analyses further supported this mechanism. For instance, targeted suppression of the heterosis-associated gene *OsMADS1* using CRISPR-Cas9 in the rice line 'Guang-liang-you 676' led to the $OsMADS1^{GW3p6}$ mutant, which exhibited significant gains in grain yield and quality [47]. *OsMADS1*, a key regulator of starch and protein metabolism [51], was functionally complemented in this mutant line, highlighting the importance of dominant gene action in enhancing complex traits. These findings underscore the critical role of heterosis and gene interactions in improving the performance of introgression lines and inform future strategies for breeding high-yielding disease-resistant cultivars.

4.4. BB Resistance in Introgression Lines

The *Xa21* gene confers BB resistance in rice through its dual role as an immune receptor, exhibiting characteristics of pattern recognition receptors (PRRs) and receptor-like kinases (RLKs) [52]. PRRs recognize conserved pathogen-associated molecular patterns (PAMPs), initiating PAMP-triggered immunity (PTI), while RLKs, specifically those containing intracellular nucleotide-binding site leucine-rich repeat (NBS-LRR) domains, mediate effector-triggered immunity (ETI) by recognizing the sulfated RaxX (required for activating *Xa21*-mediated immunity X) polypeptide secreted by *Xoo* [53]. These pathways work in concert to establish robust and specific immune responses in the host plant [54].

In this study, seven of the nine BC_5F_7 introgression lines displayed moderate resistance (MR) to BB, as evidenced by reduced lesion lengths following *Xoo* inoculation. This finding concurred with previous studies reporting that *Xa21* confers MR to various virulent *Xoo* pathotypes in different rice backgrounds including 'CB174R' [22], 'Ciherang' [26], 'HUR 917' [55], and 'RD47' [56]. The resistance observed likely reflects the coordinated action of both PTI and ETI mechanisms mediated by *Xa21* [57]. During PTI, PPR-Xa21 enhances structural barriers through lignin and cellulose deposition in xylem vessels [58], while RLK-Xa21 activates the expression of defense-related genes, including transcription factors and pathogenesis-related (PR) genes [59, 60].

Previous studies have shown that *Xa21* initiates a rapid defense response, upregulating defense gene expression within 1 to 2 hours after *Xoo* infection in the varieties 'IRBB21' and 'RD47-*Xa21*' [61]. RLK-Xa21 exhibits strong binding affinity to the RaxX protein, which is encoded by the avirulence gene *AvrXa21* in *Xoo* [21, 62]. This interaction triggers downstream defense signaling cascades, including the activation of PTI components and PR genes, ultimately contributing to enhanced BB resistance [63, 64]. RLK-Xa21 also induces the expression of positive regulators such as *OsWRKY67*, which further activate PR genes *PR1a*, *PR1b*, *PR4*, *PR10a*, and *PR10b* and reinforce resistance [65].

The effectiveness of *Xa21*-mediated resistance can be modulated by complex gene interactions. For instance, negative regulators such as *OsWRKY62*, modulated by *mitogen-activated protein kinase17* (MAPK17) can suppress *Xa21* activity, leading to reduced expression of defense genes and increased susceptibility [61]. Pyramiding *Xa21* with other BB resistance genes (*Xa4*, *xa5*, and x*a13*) elevated resistance levels by enhancing the expression of multiple defense-related genes, including *OsNPR1, OsWRKY45, OsPAL1,* and *OsPR1a* [66]. Positive regulators like *WRKY42* and *WRKY68* also play a role in strengthening *Xa21*-mediated resistance [67, 68], and the expression level and regulatory context of *Xa21* are critical for determining the robustness of BB resistance [39].

The resistance shown in six of the nine BC_5F_7 lines may also be influenced by heterozygous genomic regions on chromosomes 3, 6, 7, 8, and 9. These segments represent a mosaic of donor ('IRBB21') and recurrent ('PSL2') parent genomes and may harbor genes involved in resistance regulation or interactions affecting agronomic traits. The retained donor DNA segments—particularly on chromosome 12 in lines 'B1' and 'B2' in the BC_5F_6 generation—suggest possible linkage effects or selective advantages associated with heterozygosity. These findings concurred with previous reports that highlighted the role of heterozygous loci in conferring MR in other introgression lines [69, 70].

To further elucidate the mechanisms underlying *Xa21*-mediated resistance, complementary approaches such as transcriptomic and proteomic profiling should be employed to examine gene expression and protein activity in response to *Xoo* infection. This will clarify how the regulators *WRKY42* and *WRKY68* modulate resistance pathways and how heterozygous genomic segments contribute to trait variation. Evaluating these lines under diverse environmental conditions will provide critical insights into the stability and practical relevance of resistance and yield traits across different agroecological zones.

Our results demonstrate the success of introgression breeding by combining disease resistance and favorable agronomic traits. WGS offers precise genomic insights but integrating functional genomics and multi-environmental testing is essential to fully validate the durability and applicability of the improved lines.

5. Conclusion

The development of BC_5F_6 'PSL2-*Xa21*' rice introgression lines represents a significant advancement in breeding efforts to improve agronomic traits and resistance to BB. These lines exhibited improved plant height, grain yield, and moderate BB resistance, underscoring their potential to address major challenges in rice cultivation. WGS provided a comprehensive genetic profile, confirming a high recovery of the recurrent parent genome ('PSL2') and the successful introgression of the key resistance gene from the donor parent. The subsequent generation (BC_5F_7) retained superior agronomic traits and moderate BB resistance under greenhouse conditions, further supporting the practical utility of these lines across diverse agricultural environments. Our findings demonstrate the effectiveness of advanced backcross breeding combined with genomic tools and also emphasize broader relevance for enhancing crop productivity and resilience. This study highlighted the importance of interesting molecular tools like WGS, with complementary techniques including transcriptomics and proteomics, to validate and explore gene interactions. Such integrative approaches deepen our understanding of the biological mechanisms underlying heterosis and disease resistance, ultimately facilitating the development of more robust cultivars. The insights gained from this research contribute to a deeper understanding of introgression breeding and provide a strong foundation for developing high-yielding, disease-resistant rice varieties. The BC_5F_7 'PSL2-*Xa21*' lines offer valuable genetic resources for rice breeding programs, with the potential to support global food security and promote sustainable agriculture.

6. Declarations

6.1. Author Contributions

Conceptualization, K.S. and P.I.; methodology, K.R., T.R., K.B., K.S., and P.I.; validation, K.S. and P.I.; formal analysis, W.L.; investigation, W.L. and N.M.; resources, K.S. and P.I.; data curation, P.I.; writing—original draft preparation, W.L.; writing—review and editing, K.R., T.R., K.B., K.S., and P.I.; visualization, W.L.; supervision, K.S.; project administration, P.I.; funding acquisition, P.I. All the authors have read and agreed to the published version of the manuscript.

6.2. Data Availability Statement

The data presented in this study are available in the article.

6.3. Funding

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6.5. Institutional Review Board Statement

Not applicable.

6.6. Informed Consent Statement

Not applicable.

6.7. Declaration of Competing Interest

The authors declare that there are no conflicts of interest concerning the publication of this manuscript. Furthermore, all ethical considerations, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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