

Article

Marker-Assisted Development of Rice Line G163 Combining *Pi2*-Mediated Blast Resistance, *Xa7*-Mediated Bacterial Blight Resistance, and Favorable Grain Quality

Ruomin Wu, Zhiying Zhou, Jiayang Li, Huabin Xie, Jiafeng Wang * and Chun Chen *

National Engineering Research Center for Plant Space Breeding, South China Agricultural University, Guangzhou 510642, China

* Correspondence: jfwang@scau.edu.cn (J.W.); chchun@scau.edu.cn (C.C.)

Abstract

Rice blast and bacterial blight are two major diseases that threaten rice production world-wide. Developing rice cultivars with both disease resistance and desirable grain quality is an important objective in modern rice breeding. In this study, R1179 carrying *Pi2*, *Wx^b* and *Alk^b* was crossed with IRBB7 carrying *Xa7*, *Wx^b* and *Alk^b*. Marker-assisted selection (MAS) was applied to an F₂ population of 1123 plants, and a line, G163, combining *Pi2*, *Xa7*, *Wx^b* and *Alk^b*, was developed. Disease resistance evaluation showed that G163 retained the blast resistance conferred by *Pi2* and the bacterial blight resistance conferred by *Xa7*, exhibiting resistance to the tested isolates of both pathogens. Grain quality assessment indicated that G163 possessed favorable milling, appearance and eating quality traits. Rapid Visco Analyzer (RVA) profiling further indicated desirable starch pasting properties. In conclusion, the rice line G163 carrying *Pi2*, *Xa7*, *Wx^b* and *Alk^b* was successfully developed through MAS. The results provide a useful germplasm resource for breeding rice cultivars with combined disease resistance and desirable grain quality.

Keywords: rice; marker-assisted selection; *Pi2*; *Xa7*; blast resistance; bacterial blight resistance; grain quality

1. Introduction

Rice blast, caused by *Magnaporthe oryzae*, and bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), are among the most consequential diseases of rice. Their effects vary with cultivar, pathogen population, climate and crop management, but both diseases can substantially reduce production when susceptible cultivars encounter favorable conditions for infection [1–3]. Chemical and agronomic measures can lessen disease pressure; however, host resistance remains a central component of integrated disease management because it provides a cost-effective and environmentally sustainable approach for crop protection. Genetic improvement has become an effective and sustainable strategy for controlling rice diseases. Numerous resistance genes and quantitative resistance loci have been identified and incorporated into rice breeding programs. However, individual resistance genes generally provide protection against specific pathogens and therefore may not be sufficient to address the simultaneous occurrence of multiple major diseases in rice production systems. Therefore, combining complementary resistance genes through gene pyramiding and MAS provides an effective strategy for developing rice cultivars with enhanced resistance to multiple diseases.



Academic Editor: Meixue Zhou

Received: 1 July 2026

Revised: 27 July 2026

Accepted: 5 August 2026

Published: 6 August 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

Pi2 encodes a nucleotide-binding site leucine-rich repeat (NBS-LRR) gene on rice chromosome 6 and has been associated with resistance to multiple *M. oryzae* isolates. Molecular comparison of *Pi2* and related alleles has also shown that relatively small sequence differences can alter resistance specificity [4]. *Xa7*, likewise located on chromosome 6, confers resistance to bacterial blight through an executor-type immune mechanism activated by *Xoo* transcription activator-like (TAL) effectors [5]. *Xa7* is of particular breeding interest because its resistance has remained effective in several production contexts and can become stronger at elevated temperatures, although its performance still needs to be evaluated against the *Xoo* populations present in each target environment [5,6]. Combining *Pi2* and *Xa7* therefore offers a practical route to protect rice against two taxonomically distinct pathogens rather than relying on resistance to only one disease.

Disease resistance alone is not sufficient for cultivar adoption. Milling recovery, grain appearance and cooking and eating quality strongly influence the value of rice to producers, processors and consumers [7]. Two major starch-related loci are especially relevant to these traits. The *Waxy* (*Wx*) gene encodes granule-bound starch synthase I and is a major determinant of endosperm amylose content. The *Wx^b* allele contains a GT-to-TT substitution at the 5' splice site of the first intron, which reduces efficient transcript processing and is commonly associated with lower amylose content than the *Wx^a* allele [8,9]. The *ALK* locus encodes starch synthase IIa and contributes to variation in amylopectin structure and gelatinization temperature [10–12]. The phenotypic effects of *Wx* and *ALK* are nevertheless shaped by genetic background and grain-production conditions; consequently, favorable marker genotypes should be confirmed by direct measurements of grain quality.

MAS can improve the efficiency of combining well-characterized loci, particularly when phenotypic screening is destructive, costly or strongly affected by the environment [13]. Gene pyramiding has been used successfully in rice, including the combination of multiple bacterial blight resistance genes [14,15]. The present cross poses a specific selection problem because *Pi2*, *Xa7*, *Wx* and *ALK* are all located on chromosome 6. In R1179 × IRBB7, the two resistance genes originate from different parents, so recovery of a chromosome carrying both favorable resistance alleles depends on recombination and adequate population size. In contrast, the favorable *Wx^b* and *Alk^b* alleles are reported in both parents; screening these loci primarily confirms their retention rather than creating new allelic combinations.

In this study, R1179, carrying *Pi2*, *Wx^b* and *Alk^b*, was crossed with IRBB7, carrying *Xa7*, *Wx^b* and *Alk^b*. Gene-specific markers were used to screen 1123 F₂ plants, and selected progeny were advanced by selfing and phenotypic selection to develop the homozygous line G163. The objectives were to (i) recover and fix the target *Pi2*-*Xa7*-*Wx^b*-*Alk^b* genotype, (ii) evaluate the response of G163 to three *M. oryzae* isolates and four *Xoo* isolates, and (iii) characterize its grain quality, starch pasting properties and major agronomic traits relative to the parental lines. The work was intended to produce an intermediate breeding resource and to assess its performance under the specific experimental conditions used, rather than to establish broad adaptation or durable resistance across environments.

2. Materials and Methods

2.1. Plant Materials

The female parent used in this study was Hanghui 1179 (R1179), an indica rice blast-resistant restorer line developed by our laboratory through the cross between Hanghui 179 and Teyouzhuan, carrying the rice blast resistance gene *Pi2* and the favorable quality alleles *Wx^b* and *Alk^b*. The male parent was IRBB7, an IR24-background near-isogenic line carrying the bacterial blight resistance gene *Xa7* and the favorable quality alleles *Wx^b* and *Alk^b*, which was obtained from the National Crop Germplasm Resources Bank (Rice

Medium-Term Genebank), China. R1179 was crossed with IRBB7, and MAS was used to pyramid the blast resistance gene *Pi2* and bacterial blight resistance gene *Xa7* while retaining the favorable quality alleles *Wx^b* and *Alk^b*, resulting in the development of the rice line G163. These two parental lines have distinct genetic backgrounds and were selected as complementary parents to introduce *Pi2* and *Xa7* while retaining the favorable grain quality alleles *Wx^b* and *Alk^b*.

2.2. Rice Genomic DNA Extraction

Approximately 2–3 cm of fresh rice leaf tissue was collected and placed into a pre-chilled 2.0 mL sterile centrifuge tube. Steel beads were added, and the tissue was thoroughly ground using a 2010 Geno/Grinder® (SPEX SamplePrep, Metuchen, NJ, USA). Rice genomic DNA was extracted using the cetyltrimethylammonium bromide method as described by Pérez-Pazos et al. [16]. After quality assessment, the extracted DNA was dissolved in sterile ddH₂O and stored at −20 °C until use.

2.3. Molecular Marker Design and Genotyping

Gene-specific molecular markers were developed based on the sequence information of *Pi2*, *Xa7*, *Wx^b*, and *Alk^b*. The primer sequences are listed in Table 1. Among them, *Pi2* was detected using a Kompetitive Allele Specific PCR (KASP) marker, *Xa7* was identified using a codominant PCR marker, and the genotypes of *Wx^b* and *Alk^b* were determined by sequencing following specific PCR amplification.

Table 1. Molecular markers and sequencing primers used for target genes.

Gene	Marker	Primer Sequence (5′–3′)	Reference
<i>Pi2</i>	Pi2-F1/T	GAAGGTGACCAAGTTCATGCTCCTACCATCCCATCTGAAACCATGT	[17]
	Pi2-F2/C Pi2-R	GAAGGTCGGAGTCAACGGATTCTACCATCCCATCTGAAACCATGC AACAAACCCCTTCTGCTATCCATCT	
<i>Xa7</i>	Xa7-F	AGCCCTGACTGCTAAAACCA	[18]
	Xa7-R Xa7null-F Xa7null-R	GCTCTTCAAGTGTGCGATGC AACCGAGGGAGGTGATTTC GTACATCATTGTCCCCACGG	
<i>Wx^b</i>	Wx-CX-F Wx-CX-R	GAACAGAAGGGCCCTGACGT ATCTCATCCCCTGCAGCTGG	This study
<i>Alk^b</i>	Alk-CX-F Alk-CX-R	CCGGAAGCTAATTGCGCACC GTTTCGAGGCGCAGCACACA	This study

2.4. PCR Amplification and Gel Electrophoresis Analysis

The total volume of the PCR reaction was 20 µL, consisting of 1 µL template DNA, 1 µL forward primer, 1 µL reverse primer, 10 µL 2× PCR Mix, and sterile ddH₂O added to a final volume of 20 µL. The PCR amplification program was adjusted according to the annealing temperature of the primers and the size of the target fragment. In general, the amplification conditions were as follows: initial denaturation at 95 °C for 5 min; followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C (adjusted as appropriate for different primers) for 30 s, and extension at 72 °C for 1 min; with a final extension at 72 °C for 5 min and storage at 4 °C. PCR products were separated by electrophoresis on a 3% agarose gel and visualized using a gel imaging system (Tanon 2500R, Tanon Science & Technology Co., Ltd., Shanghai, China).

2.5. KASP Genotyping Assay

The *Pi2* gene was genotyped using Kompetitive Allele Specific PCR (KASP) technology. The total KASP reaction volume was 10 µL, including genomic DNA template, KASP

primer mixture, and KASP Master Mix. PCR amplification was performed according to the standard KASP protocol: initial denaturation at 95 °C for 15 min; followed by 10 cycles of 95 °C for 20 s and annealing from 61 °C to 55 °C for 60 s, with the annealing temperature decreasing by 0.6 °C per cycle; followed by 26 cycles of 95 °C for 20 s and 55 °C for 60 s. After amplification, FAM and HEX fluorescence signals were detected using a FLUOstar Omega SNP microplate reader (BMG LABTECH, Ortenberg, Germany). Genotypes were determined based on the clustering positions of samples in the scatter plot and were classified as the homozygous R1179 type, heterozygous type, or homozygous IRBB7 type.

2.6. Evaluation of Rice Blast Resistance

Three rice blast isolates (GDYJ7, GUY11, and ZJ21X18) were used for disease resistance evaluation. GUY11 is a widely used model isolate of *M. oryzae*. GDYJ7 and ZJ21X18 were isolated from diseased rice samples collected in Guangdong and Zhejiang provinces, respectively, by our laboratory. One hundred seeds of each test material were soaked, germinated, and sown. When the seedlings reached the three-leaf-one-heart stage, spore suspensions were prepared from the corresponding isolates at a concentration of 5×10^5 spores mL⁻¹. Detached-leaf inoculation was performed according to the method described by Chuanchao et al. [17]. Three leaves were inoculated for each material, and two inoculation sites were made on each leaf. At 7 d after inoculation, lesion phenotypes were photographed and lesion lengths were measured. Disease resistance was evaluated by comparing the lesion lengths of the tested materials with those of the parental lines. In this study, IRBB7, which lacks the *Pi2* resistance gene, was used as a susceptible reference control in the blast resistance evaluation.

2.7. Evaluation of Bacterial Blight Resistance

Four *Xoo* isolates (GDXO-1, PXO61, PXO99A, and GU2001) were used for bacterial blight resistance evaluation. PXO61 and PXO99A are well-characterized *Xoo* strains originally isolated from the Philippines and provided by Professor Yongqiang He's laboratory at Guangxi University. GU2001 is a Guangxi-derived *Xoo* strain provided by the same laboratory. GDXO-1 was isolated from a diseased rice sample collected in Guangdong by our laboratory. The bacterial isolates were cultured on nutrient agar (NA) medium at 28 °C for 2 d. Bacterial suspensions were prepared in phosphate-buffered saline (PBS) and adjusted to an optical density of OD₆₀₀ = 1.0. At the booting stage, plants were inoculated using the leaf-clipping method. The tips (approximately 1–2 cm) of the uppermost four to five fully expanded leaves of each plant were clipped with scissors dipped in the bacterial suspension. Disease symptoms were evaluated 21 d after inoculation by measuring lesion lengths, which were used as the criterion for resistance assessment. In this study, R1179, which lacks the *Xa7* resistance gene, was used as a susceptible reference control in the bacterial blight resistance evaluation.

2.8. Grain Quality Evaluation

Mature seeds of G163 and the parental lines R1179 and IRBB7 were harvested, air-dried, and stored under cool and ventilated conditions for three months to allow stabilization of their physicochemical properties before grain quality evaluation. Grain quality traits were determined according to the Chinese National Standard GB/T 17891-2017 [19]. The evaluated parameters included brown rice rate (BRR), milled rice rate (MRR), head rice rate (HRR), amylose content (AC), gel consistency (GC), alkali spreading value (ASV), and chalkiness degree.

For all samples, well-filled mature grains were uniformly milled prior to analysis. Starch pasting properties were determined using a Rapid Visco Analyzer (RVA; TecMaster,

Perten Instruments, Stockholm, Sweden). Rice flour was ground and passed through a 100-mesh sieve. Based on a moisture content of 12%, 3.00 g of rice flour was weighed and mixed with 25.00 mL of distilled water in an RVA canister. The RVA profile was measured using the following program: holding at 50 °C for 1 min, heating to 95 °C at a rate of 12 °C min⁻¹ (3.75 min), holding at 95 °C for 2 min, cooling to 50 °C at the same rate (3.75 min), and finally holding at 50 °C for 1.4 min. The paddle rotated at 960 rpm during the first 10 s and then at 160 rpm for the remainder of the test. The RVA parameters obtained from the viscosity profile included peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BDV), final viscosity (FV), setback viscosity (SBV), peak time (PT), and pasting temperature (PTemp).

2.9. Agronomic Trait Evaluation and Statistical Analysis

G163 and its parental lines, R1179 and IRBB7, were grown at the Qilinbei Experimental Farm of South China Agricultural University, Guangzhou, China (23.16° N, 113.36° E). Plants were transplanted at a spacing of 20 cm × 20 cm. Only one plot per genotype was used in this field evaluation. Each plot consisted of six rows with six plants per row and was managed according to standard agronomic practices. Each material was planted in a single plot, and each plot consisted of six rows with six plants per row. The field management followed standard agronomic practices.

At maturity, three plants with uniform growth were randomly selected from each plot for agronomic trait evaluation, representing three sampled plants from the single plot of each genotype. The investigated traits included plant height, flag leaf length, flag leaf width, grain length, grain width, effective tiller number, panicle length, total grain number per panicle, seed-setting rate, and 1000-grain weight.

All data were analyzed using GraphPad Prism 5 software and are presented as the mean ± standard deviation (mean ± SD). Differences among materials for lesion length, grain quality traits, RVA parameters, and agronomic traits were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Different lowercase letters indicate significant differences among materials at $p < 0.05$.

3. Results

3.1. Marker-Assisted Pyramiding of *Pi2* and *Xa7* While Retaining *Wx^b* and *Alk^b*

To develop a rice line combining blast resistance, bacterial blight resistance, and desirable grain quality traits, R1179, carrying the blast resistance gene *Pi2* and the favorable quality alleles *Wx^b* and *Alk^b*, was crossed with IRBB7, carrying the bacterial blight resistance gene *Xa7* and the favorable quality alleles *Wx^b* and *Alk^b*. MAS was subsequently applied to the segregating population using molecular markers linked to the target genes (Figure 1).

Because *Pi2*, *Xa7*, *Wx*, and *Alk* are all located on chromosome 6, and *Pi2* and *Xa7* originated from different parental lines, pyramiding of the target genes depended on effective recombination between homologous chromosomes. Genotyping of the F₁ plants confirmed the presence of target alleles from both parents, indicating successful hybridization and suitability for subsequent population development and genotype selection. In the F₂ population consisting of 1123 individuals, the four target loci, *Pi2*, *Xa7*, *Wx^b*, and *Alk^b*, were simultaneously screened using molecular markers. Three homozygous plants carrying *Pi2* and *Xa7* together with the retained favorable alleles *Wx^b* and *Alk^b* were recovered from 1123 F₂ individuals and subsequently advanced through selfing and MAS. These plants represented the selected progeny carrying the desired allele combination rather than the total number of recombinant individuals between *Pi2* and *Xa7*.

The selected plants were subsequently self-pollinated from the F₃ to F₆ generations and continuously monitored through molecular marker analysis and field-based agronomic

selection. The target genotypes were stably inherited in the selected lines, while plant architecture, fertility, and major agronomic traits gradually became uniform. Ultimately, a rice line carrying *Pi2*, *Xa7*, *Wx^b*, and *Alk^b* with favorable agronomic performance was obtained in the F₆ generation and designated as G163.

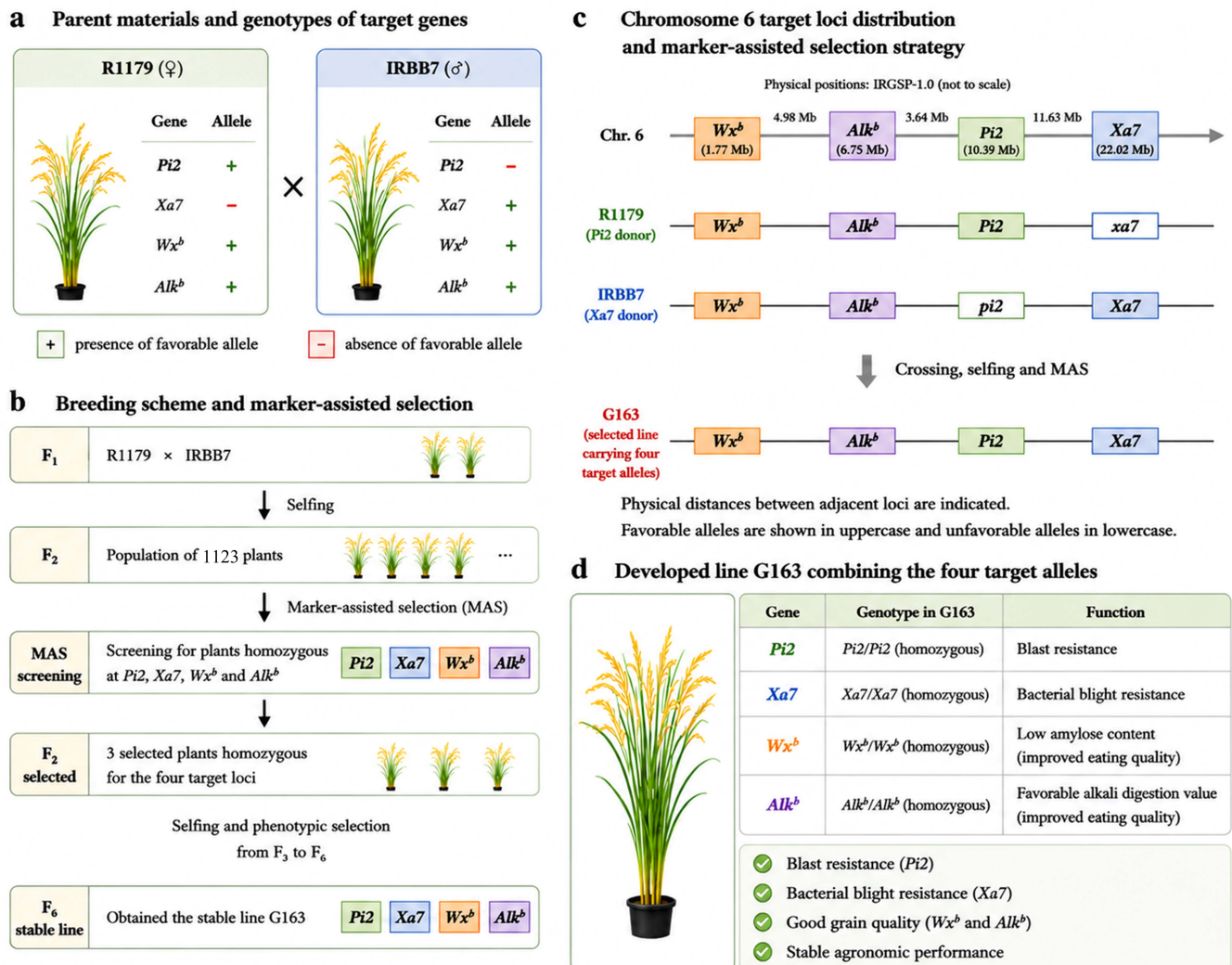


Figure 1. Development of rice line G163 combining blast resistance, bacterial blight resistance, and favorable grain quality alleles through marker-assisted selection. (a) Parental materials and genotypes of the target loci. R1179 carries the favorable alleles *Pi2*, *Wx^b*, and *Alk^b*, whereas IRBB7 carries the favorable allele *Xa7*. (b) Breeding scheme and marker-assisted selection strategy used for developing G163. Three F₂ plants homozygous for the four target loci were selected and subsequently advanced through selfing and phenotypic selection from F₃ to F₆ generations. (c) Distribution of the target loci on chromosome 6 and marker-assisted allele assembly. The physical positions and distances between adjacent loci are indicated. (d) Genotypic composition and functional characteristics of the developed line G163 carrying the four target alleles.

3.2. Blast Resistance of G163

The blast resistance of G163 and its parental lines was evaluated using a detached-leaf inoculation assay. As shown in Figure 2, G163 exhibited a high level of resistance to all three rice blast isolates (GDYJ7, GUY11, and ZJ21X18). No significant differences in lesion length were observed between G163 and the *Pi2*-carrying parent R1179, whereas both showed significantly shorter lesions than the non-*Pi2* parent IRBB7.

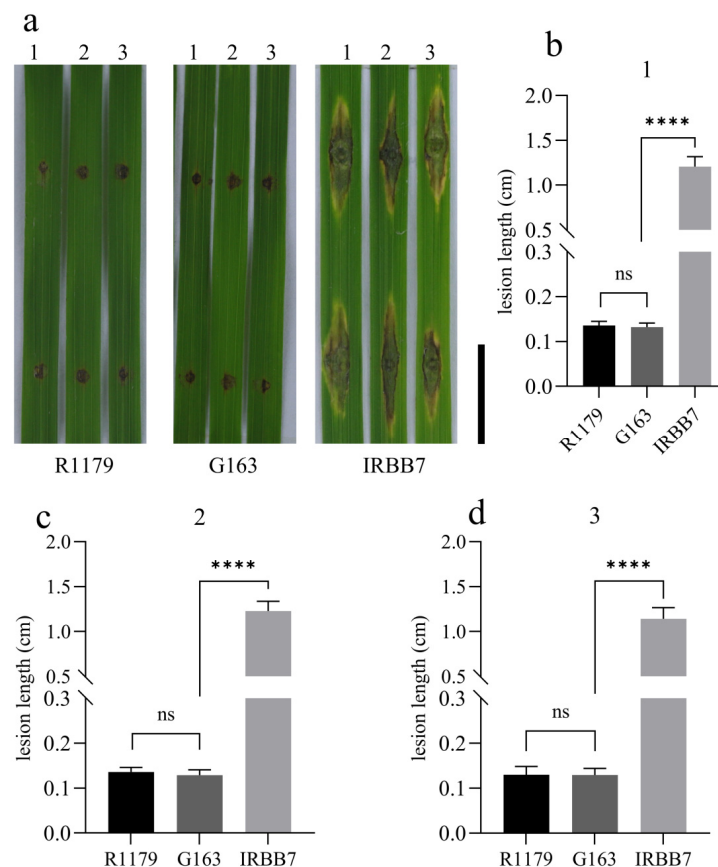


Figure 2. Blast resistance of R1179, G163 and IRBB7 inoculated with different *M. oryzae* isolates. (a) Representative lesion phenotypes of R1179, G163 and IRBB7 after inoculation with three *M. oryzae* isolates. 1, GDYJ7; 2, GUY11; 3, ZJ21X18. Scale bar = 1 cm. (b–d) Lesion length of R1179, G163 and IRBB7 inoculated with GDYJ7 (b), GUY11 (c) and ZJ21X18 (d), respectively. Data are presented as mean $\pm$ SD. Statistical significance is indicated as ns and **** above the bars. ns, not significant; ****, $p < 0.0001$.

Consistent with the lesion phenotypes, only small and restricted lesions developed on the leaves of R1179 and G163 following inoculation, whereas IRBB7 exhibited typical expanding lesions with significantly greater lesion lengths. Statistical analysis showed that lesion lengths of G163 remained at a low level under inoculation with all three isolates and were comparable to those of R1179. In contrast, lesion lengths of IRBB7 were significantly greater than those of G163 and R1179.

These results indicate that the pyramided line G163 retained the blast resistance conferred by *Pi2* and exhibited resistance to all three tested rice blast isolates. The results further suggest that the resistance conferred by *Pi2* was maintained after gene pyramiding.

3.3. Bacterial Blight Resistance of G163

Bacterial blight resistance of G163 and its parental lines was evaluated using the leaf-clipping inoculation method. As shown in Figure 3, G163 exhibited a high level of resistance to all four *Xoo* isolates (GDXO-1, PXO61, PXO99A, and GU2001). No significant differences in lesion length were observed between G163 and the *Xa7*-carrying parent IRBB7, whereas both showed significantly shorter lesions than the non-*Xa7* parent R1179.

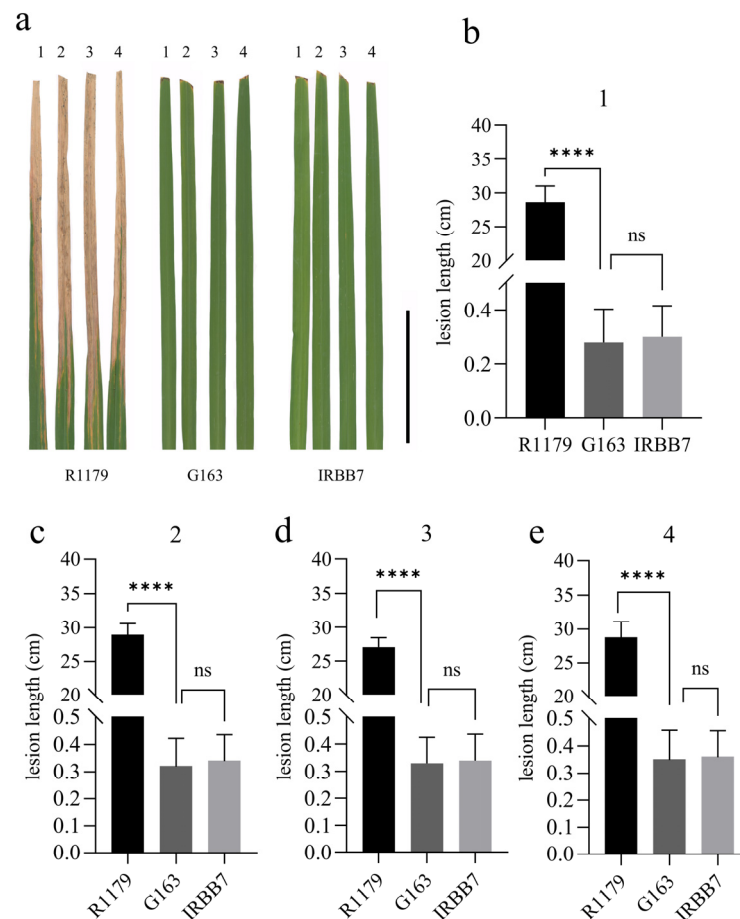


Figure 3. Resistance responses of R1179, G163 and IRBB7 to four *Xoo* isolates. (a) Representative lesion phenotypes of R1179, G163 and IRBB7 after inoculation with four *Xoo* isolates. 1, GDXO-1; 2, PXO61; 3, PXO99A; 4, GU2001. Scale bar = 10 cm. (b–e) Lesion length of R1179, G163 and IRBB7 inoculated with GDXO-1 (b), PXO61 (c), PXO99A (d) and GU2001 (e), respectively. Data are presented as mean $\pm$ SD. Statistical significance is indicated as ns and **** above the bars. ns, not significant; ****, $p < 0.0001$.

The inoculation phenotypes were consistent with the lesion length data. In R1179, lesions rapidly expanded along the leaf veins, resulting in extensive necrotic areas. In contrast, lesion expansion was markedly restricted in G163 and IRBB7, with only short lesions developing near the clipping sites. Statistical analysis further confirmed that lesion lengths of G163 were comparable to those of IRBB7 and significantly lower than those of R1179 across all four isolates.

These results indicate that G163 retained the bacterial blight resistance conferred by *Xa7* and exhibited resistance to all four tested isolates. Together with the blast resistance evaluation, these findings demonstrate that G163 successfully pyramided the resistance genes *Pi2* and *Xa7*, conferring resistance to both rice blast and bacterial blight.

3.4. Grain Quality Performance of G163

In addition to the pyramided disease resistance genes, G163 also carried the favorable grain quality alleles *Wx^b* and *Alk^b*. The grain appearance and quality characteristics of G163 are shown in Figure 4. To evaluate its grain quality performance, the major quality traits of G163 and its parental lines were systematically analyzed (Table 2).

For cooking and eating quality, G163 exhibited an amylose content within the desirable range for high-quality indica rice. Its gel consistency was significantly higher than that of IRBB7, whereas no significant differences in alkali spreading value were observed among

the three materials. Regarding milling quality, G163 showed a significantly higher brown rice rate, milled rice rate, and head rice rate than both parental lines. Notably, the head rice rate of G163 was significantly higher than that of IRBB7, indicating improved milling performance. For appearance quality, G163 exhibited significantly lower chalkiness than IRBB7 and was comparable to R1179. Overall, G163 displayed favorable appearance quality characterized by low chalkiness.

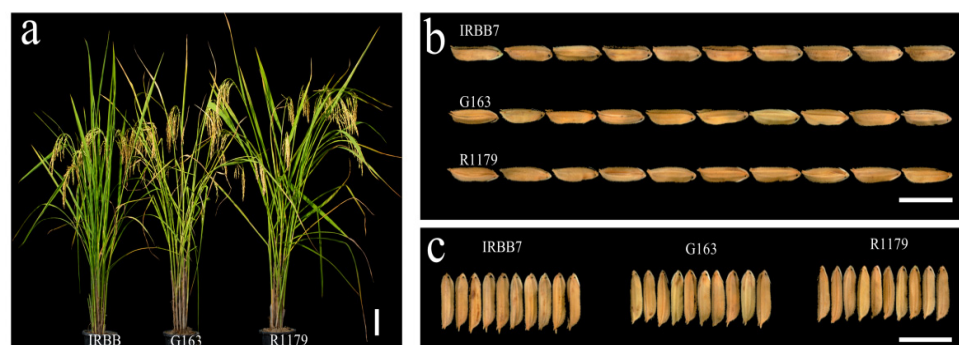


Figure 4. Plant architecture and grain morphology of R1179, G163 and IRBB7. (a) Plant architecture of R1179, G163 and IRBB7 at the mature stage. Scale bar = 10 cm. (b) Comparison of grain length among R1179, G163 and IRBB7. Scale bar = 1 cm. (c) Comparison of grain width among R1179, G163 and IRBB7. Scale bar = 1 cm.

Table 2. Grain quality traits of G163 and its parental lines.

Material	BRR (%)	MRR (%)	HRR (%)	AC (%)	GC (mm)	ASV	CD (%)
R1179	75.42 ± 0.94 b	61.16 ± 0.70 b	47.09 ± 0.40 b	15.30 ± 0.08 a	61.86 ± 3.11 b	6.00 ± 0.00 a	0.62 ± 0.39 b
IRBB7	74.97 ± 0.49 b	60.87 ± 0.17 b	42.71 ± 0.49 c	14.58 ± 0.17 b	45.58 ± 3.13 c	6.33 ± 0.58 a	8.30 ± 1.41 a
G163	78.18 ± 0.54 a	63.25 ± 0.11 a	52.79 ± 0.53 a	14.61 ± 0.11 b	70.88 ± 1.85 a	6.67 ± 0.58 a	0.80 ± 0.32 b

Note: BRR, brown rice rate; MRR, milled rice rate; HRR, head rice rate; AC, amylose content; GC, gel consistency; ASV, alkali spreading value; CD, chalkiness degree. Values are presented as mean ± SD ($n = 3$). Different lowercase letters within a column indicate significant differences among materials according to Tukey's multiple comparison test ($p < 0.05$).

To further evaluate the cooking and eating quality of G163, its starch pasting properties were analyzed using RVA (Table 3). Clear differences in RVA parameters were observed among the tested materials. Compared with the parental lines, G163 exhibited lower peak viscosity, trough viscosity, and breakdown viscosity, indicating a relatively lower degree of starch granule swelling during heating.

Table 3. RVA profile parameters of G163 and its parental lines.

Material	PV (RVU)	TV (RVU)	BDV (RVU)	FV (RVU)	SBV (RVU)	PT (min)	PTemp (°C)
R1179	3503.67 ± 15.01 a	2219.33 ± 15.14 a	1284.33 ± 10.26 a	3590.00 ± 28.16 a	86.33 ± 15.31 b	5.27 ± 0.07 b	78.33 ± 0.06 b
IRBB7	3478.00 ± 102.31 a	2306.33 ± 90.90 a	1171.67 ± 30.92 b	3582.00 ± 94.49 a	104.00 ± 16.52 a	6.00 ± 0.07 a	80.45 ± 0.44 a
G163	3129.67 ± 2.08 b	2168.00 ± 1.73 b	961.67 ± 1.53 c	3181.00 ± 5.29 b	51.33 ± 3.79 c	4.89 ± 0.04 c	80.63 ± 0.08 a

Note: PV, peak viscosity; TV, trough viscosity; BDV, breakdown viscosity; FV, final viscosity; SBV, setback viscosity; PT, peak time; PTemp, pasting temperature. Values are presented as mean ± SD ($n = 3$). Different lowercase letters within a column indicate significant differences among materials according to Tukey's multiple comparison test ($p < 0.05$).

Notably, the setback viscosity (SBV) of G163 was only 51.33 RVU, markedly lower than those of both R1179 and IRBB7, suggesting a lower tendency for starch retrogradation during cooling. In addition, G163 reached peak viscosity more rapidly than the parental lines, indicating a relatively faster gelatinization process. The pasting temperature of G163 was similar to that of IRBB7 but higher than that of R1179. Combined with the results of amylose content, gel consistency, and alkali spreading value, the relatively low SBV and high gel consistency of G163 suggest favorable cooking and eating quality.

Taken together, the grain quality analyses demonstrated that G163 combined desirable cooking and eating quality, milling quality, and appearance quality while maintaining resistance to both rice blast and bacterial blight. These results suggest that G163 is a promising intermediate breeding line for developing rice cultivars with both disease resistance and desirable grain quality and may serve as a valuable germplasm resource for rice improvement.

3.5. Agronomic Performance of G163

The agronomic traits of G163 and its parental lines were evaluated and the results are presented in Table 4 and Figure 4. Significant differences were observed among the tested materials under the experimental conditions for plant height, flag leaf length, total grain number per panicle, and 1000-grain weight, whereas no significant differences were detected for effective tiller number, flag leaf width, panicle length, seed-setting rate, or grain shape-related traits. G163 exhibited an intermediate plant height compared with its parental lines. The effective tiller number and panicle length of G163 were comparable to those of both parents. The total grain number per panicle of G163 was significantly higher than that of IRBB7 and comparable to that of R1179. In addition, G163 showed a significantly higher 1000-grain weight than both parental lines. No significant differences were observed among the three materials for seed-setting rate, grain length, or grain width. Overall, G163 showed generally comparable agronomic performance under the tested conditions while pyramiding disease resistance and grain quality genes. In particular, G163 showed improved 1000-grain weight while maintaining other agronomic traits comparable to those of its parental lines.

Table 4. Agronomic traits of G163 and its parental lines.

Material	Plant Height (cm)	Effective Tiller Number	Flag Leaf Length (cm)	Flag Leaf Width (cm)	Panicle Length (cm)	Total Grain Number per Panicle	Seed-Setting Rate (%)	1000-Grain Weight (g)	Grain Length (mm)	Grain Width (mm)
G163	99.17 ± 1.14 b	8.70 ± 0.95 a	38.35 ± 2.27 b	2.03 ± 0.05 a	23.60 ± 1.17 a	165.90 ± 12.70 a	88.57 ± 1.87 a	21.58 ± 0.15 a	9.34 ± 0.08 a	2.67 ± 0.02 a
R1179	103.47 ± 1.54 a	8.60 ± 0.70 a	41.19 ± 1.24 a	2.04 ± 0.05 a	24.48 ± 1.08 a	163.20 ± 8.13 a	88.89 ± 1.61 a	21.30 ± 0.10 b	9.36 ± 0.09 a	2.65 ± 0.03 a
IRBB7	96.50 ± 1.43 c	8.70 ± 0.48 a	39.16 ± 2.30 ab	2.04 ± 0.05 a	24.52 ± 0.92 a	144.20 ± 7.83 b	88.57 ± 1.58 a	21.32 ± 0.14 b	9.42 ± 0.09 a	2.64 ± 0.02 a

Note: Values are presented as mean ± SD ($n = 3$ individual plants). Different lowercase letters indicate significant differences among materials according to one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

4. Discussion

4.1. Feasibility of Pyramiding Target Genes Located on the Same Chromosome

Pyramiding resistance genes is an effective strategy for improving the breadth and durability of disease resistance in rice. When target genes are located on different chromosomes, their selection can largely follow independent segregation. In contrast, genes located on the same chromosome require more careful population design because the recovery of the desired genotype depends on genetic distance, local recombination frequency, and the precision of molecular markers. This situation may increase the difficulty of early-generation selection, especially when the target loci are surrounded by duplicated sequences or resistance-gene clusters. However, same-chromosome pyramiding also has a practical advantage. Once a favorable linkage phase is obtained, the target genes can be transmitted as a relatively stable chromosomal segment, which reduces the probability of losing one of the genes during subsequent selection and facilitates the use of this segment as a breeding module [20–22].

In the present study, *Pi2* and *Xa7* were pyramided in the same genetic background. Both genes are located on rice chromosome 6, but they confer resistance to different pathogens and represent different types of resistance mechanisms. *Pi2* is a broad-spectrum blast resistance gene encoding an NBS-LRR protein [4,23]. *Xa7* confers resistance to bacterial blight and has been characterized as an executor-type resistance gene associated with TAL effector-triggered resistance [5]. The successful development of G163 indicates that the simultaneous introgression of *Pi2* and *Xa7* is genetically feasible, even though both target genes are located on the same chromosome. This result also suggests that the *Pi2*-*Xa7* combination can be fixed and subsequently transferred as a useful resistance segment in molecular breeding.

4.2. Compatibility of *Pi2* and *Xa7* in the Same Genetic Background

The breeding value of resistance gene pyramiding depends not only on the presence of target genes but also on their functional compatibility in the same genetic background. Plant immune responses may involve defense signaling, reactive oxygen species accumulation, programmed cell death, and resource allocation between growth and defense. In some cases, the accumulation or inappropriate expression of resistance genes can lead to growth penalties or unstable phenotypes [24–26]. Therefore, it is necessary to evaluate whether *Pi2* and *Xa7* can function together without obvious negative interaction.

Pi2 and *Xa7* are expected to be compatible because they respond to different types of pathogens and are involved in different recognition systems. *Pi2* mediates resistance to the fungal pathogen *M. oryzae*, whereas *Xa7* confers resistance to the bacterial pathogen *Xoo*. Thus, the two genes are unlikely to compete directly for the same pathogen-recognition process. Previous studies have shown that *Pi2* and related blast resistance genes can provide broad-spectrum resistance to rice blast, while executor-type bacterial blight resistance genes can be highly effective when corresponding TAL effectors are present in the pathogen population [4,5,23,27]. The resistance performance of G163 suggests that *Pi2* and *Xa7* can coexist in one genetic background and retain their respective resistance functions.

Nevertheless, compatibility should not be judged solely from marker genotypes. The effectiveness of blast and bacterial blight resistance genes can vary according to pathogen population structure, environmental conditions, and host genetic background. *M. oryzae* populations may overcome single major resistance genes through effector variation, while *Xa7*-mediated resistance depends on the presence and activity of corresponding TAL effectors in bacterial blight pathogens [5,28]. Therefore, G163 should be evaluated using diverse blast isolates and bacterial blight strains, and its agronomic performance should be examined under different environments. Such evaluation is important for determining whether the *Pi2*-*Xa7* segment causes linkage drag or affects yield-related traits. If stable resistance and acceptable agronomic performance are maintained, the compatibility of *Pi2* and *Xa7* in G163 would support its further application in rice disease-resistance breeding.

4.3. Application Potential of G163 in Molecular Design Breeding

G163 has potential value as an intermediate donor line for molecular design breeding. Modern rice improvement increasingly requires the simultaneous optimization of disease resistance, yield, grain quality, and regional adaptation. In this context, a line carrying a fixed *Pi2*-*Xa7* combination provides a ready-to-use resistance module against two important rice diseases. Compared with using separate donors for blast and bacterial blight resistance, G163 may simplify crossing design, reduce the number of selection generations, and may facilitate the development of rice cultivars with combined resistance.

In practical breeding, G163 can be used in marker-assisted backcrossing to improve elite but disease-susceptible varieties. Foreground selection can be applied to retain *Pi2*

and *Xa7*, while background selection can accelerate the recovery of the recurrent parent genome and reduce unwanted donor segments [29–31]. G163 may also be used as a parent in multi-gene pyramiding schemes, in which the *Pi2-Xa7* segment is combined with additional genes or quantitative resistance loci according to the disease pressure of the target region. Such an approach is consistent with current breeding strategies that integrate MAS, high-throughput genotyping, and multi-environment phenotypic evaluation [32–34].

The application of G163 should be accompanied by precise molecular markers and repeated resistance evaluation. Functional or tightly linked markers for *Pi2* and *Xa7* would allow rapid identification of target genotypes in large segregating populations. At the same time, artificial inoculation and field disease nursery tests remain necessary because molecular markers cannot fully represent resistance performance under variable pathogen and environmental conditions. With the integration of MAS, background recovery analysis, and multi-environment disease evaluation, G163 can serve as a useful genetic resource for developing rice lines with combined resistance to blast and bacterial blight [34,35].

However, several limitations should be considered in this study. The field evaluation was conducted at a single location (Guangzhou) and in a single growing season, and each material was planted in only one plot. Agronomic traits were evaluated using three representative plants from each plot. Because grain quality traits, disease resistance, and agronomic performance can be influenced by environmental conditions, the limited sampling size and lack of replicated field trials may reduce the statistical power of comparisons among genotypes. Although G163 exhibited favorable disease resistance, grain quality, and agronomic performance under the tested conditions, further validation using larger sample sizes, replicated field trials, and multi-environment experiments is required to confirm its stability and breeding potential.

In addition, G163 was developed through conventional crossing combined with MAS, and selection was performed primarily based on the target loci *Pi2*, *Xa7*, *Wx^b*, and *Alk^b*. Although the selected plants were confirmed to carry the desired allelic combination at these target loci, recombination of other genomic regions between the parental lines may have occurred during segregation. Since genome-wide background analysis was not performed in this study, the potential contribution of additional recombinant genomic segments to the observed agronomic traits and disease resistance performance of G163 cannot be completely excluded. Future whole-genome background analysis and multi-environment evaluation will help further clarify the genetic basis and breeding value of G163.

5. Conclusions

MAS in a population of 1123 F₂ plants enabled the recovery of progeny carrying the target *Pi2*, *Xa7*, *Wx^b* and *Alk^b* alleles and the development of the homozygous line G163. Under the conditions tested, G163 showed short lesions in response to three *M. oryzae* isolates and four *Xoo* isolates, while maintaining favorable milling and appearance traits, an amylose content of about 14.6%, high gel consistency and distinct starch pasting characteristics. Its major agronomic traits were generally comparable to those of the parental lines. These findings support the feasibility of using MAS to recover linked resistance loci while retaining shared favorable quality alleles on chromosome 6. G163 therefore represents a potentially useful intermediate donor for breeding rice with combined blast and bacterial blight resistance and acceptable grain quality. However, further validation using larger sample sizes, replicated field trials, and multi-environment experiments will be required to confirm its agronomic stability, resistance stability, adaptation, and breeding value.

Author Contributions: Conceptualization, R.W., Z.Z., J.W. and C.C.; Methodology, R.W. and Z.Z.; Software, R.W. and Z.Z.; Formal analysis, R.W., Z.Z., J.W. and C.C.; Investigation, R.W. and Z.Z.; Data curation, R.W., Z.Z., J.L. and H.X.; Writing—original draft, R.W., Z.Z., J.L. and H.X.; Writing—review

and editing, J.L. and H.X.; Visualization, R.W.; Supervision, J.W. and C.C.; Funding acquisition, J.W. and C.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Heyuan Dengta Basin Seed Industry Base Construction Project—Construction of the Rice Space Mutagenesis Breeding Base (Grant No. YCN2025-162), and the National Key Research and Development Plan (Grant Nos. 2022YFD1200703-01 and 2023YFD1400205-1), China.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Savary, S.; Willocquet, L.; Pethybridge, S.J.; Esker, P.; McRoberts, N.; Nelson, A. The global burden of pathogens and pests on major food crops. *Nat. Ecol. Evol.* **2019**, *3*, 430–439. [[CrossRef](#)] [[PubMed](#)]
2. Dean, R.; Van Kan, J.A.; Pretorius, Z.A.; Hammond Kosack, K.E.; Di Pietro, A.; Spanu, P.D.; Rudd, J.J.; Dickman, M.; Kahmann, R.; Ellis, J. The Top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* **2012**, *13*, 414–430. [[CrossRef](#)] [[PubMed](#)]
3. Niño Liu, D.O.; Ronald, P.C.; Bogdanove, A.J. *Xanthomonas oryzae* pathovars: Model pathogens of a model crop. *Mol. Plant Pathol.* **2006**, *7*, 303–324. [[CrossRef](#)] [[PubMed](#)]
4. Zhou, B.; Qu, S.; Liu, G.; Dolan, M.; Sakai, H.; Lu, G.; Bellizzi, M.; Wang, G. The eight amino-acid differences within three leucine-rich repeats between Pi2 and Piz-t resistance proteins determine the resistance specificity to *Magnaporthe grisea*. *Mol. Plant. Microbe. Interact.* **2006**, *19*, 1216–1228. [[CrossRef](#)] [[PubMed](#)]
5. Chen, X.; Liu, P.; Mei, L.; He, X.; Chen, L.; Liu, H.; Shen, S.; Ji, Z.; Zheng, X.; Zhang, Y. Xa7, a new executor R gene that confers durable and broad-spectrum resistance to bacterial blight disease in rice. *Plant Commun.* **2021**, *2*, 100143. [[CrossRef](#)] [[PubMed](#)]
6. Webb, K.M.; Ona, I.; Bai, J.; Garrett, K.A.; Mew, T.; Vera Cruz, C.M.; Leach, J.E. A benefit of high temperature: Increased effectiveness of a rice bacterial blight disease resistance gene. *New Phytol.* **2010**, *185*, 568–576. [[PubMed](#)]
7. Fitzgerald, M.A.; McCouch, S.R.; Hall, R.D. Not just a grain of rice: The quest for quality. *Trends Plant Sci.* **2009**, *14*, 133–139. [[CrossRef](#)] [[PubMed](#)]
8. Wang, Z.Y.; Zheng, F.Q.; Shen, G.Z.; Gao, J.P.; Snustad, D.P.; Li, M.G.; Zhang, J.L.; Hong, M.M. The amylose content in rice endosperm is related to the post-transcriptional regulation of the *waxy* gene. *Plant J.* **1995**, *7*, 613–622. [[CrossRef](#)] [[PubMed](#)]
9. Isshiki, M.; Morino, K.; Nakajima, M.; Okagaki, R.J.; Wessler, S.R.; Izawa, T.; Shimamoto, K. A naturally occurring functional allele of the rice *waxy* locus has a GT to TT mutation at the 5' splice site of the first intron. *Plant J.* **1998**, *15*, 133–138. [[CrossRef](#)] [[PubMed](#)]
10. Gao, Z.; Zeng, D.; Cui, X.; Zhou, Y.; Yan, M.; Huang, D.; Li, J.; Qian, Q. Map-based cloning of the *ALK* gene, which controls the gelatinization temperature of rice. *Sci. China Ser. C Life Sci.* **2003**, *46*, 661–668. [[CrossRef](#)] [[PubMed](#)]
11. Umemoto, T.; Aoki, N.; Lin, H.; Nakamura, Y.; Inouchi, N.; Sato, Y.; Yano, M.; Hirabayashi, H.; Maruyama, S. Natural variation in rice starch synthase IIa affects enzyme and starch properties. *Funct. Plant Biol.* **2004**, *31*, 671–684. [[CrossRef](#)] [[PubMed](#)]
12. Bao, J.S.; Corke, H.; Sun, M. Nucleotide diversity in *starch synthase IIa* and validation of single nucleotide polymorphisms in relation to starch gelatinization temperature and other physicochemical properties in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* **2006**, *113*, 1171–1183. [[CrossRef](#)] [[PubMed](#)]
13. Collard, B.C.; Mackill, D.J. Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philos. Trans. R. Soc. B Biol. Sci.* **2007**, *363*, 557–572. [[CrossRef](#)] [[PubMed](#)]
14. Huang, N.; Angeles, E.R.; Domingo, J.; Magpantay, G.; Singh, S.; Zhang, G.; Kumaravadivel, N.; Bennett, J.; Khush, G.S. Pyramiding of bacterial blight resistance genes in rice: Marker-assisted selection using RFLP and PCR. *Theor. Appl. Genet.* **1997**, *95*, 313–320. [[CrossRef](#)]
15. Singh, S.; Sidhu, J.S.; Huang, N.; Vikal, Y.; Li, Z.; Brar, D.S.; Dhaliwal, H.S.; Khush, G.S. Pyramiding three bacterial blight resistance genes (*xa5*, *xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR106. *Theor. Appl. Genet.* **2001**, *102*, 1011–1015. [[CrossRef](#)]
16. Pérez-Pazos, J.V.; Romero-Ferrer, J.L.; Berdugo-Cely, J.A. Selection of a CTAB protocol for high-quality DNA extraction in *Oryza sativa* L. Validated for application in genotyping process based on Illumina sequencing. *J. Crop Sci. Biotechnol.* **2023**, *26*, 433–446.
17. Chuanchao, Z.; Sen, C.; Siyi, L.; Huabin, X.; Wenyu, L.; Wuming, X.; Ming, H.; Tao, G.; Jiafeng, W. Development and application of KASP marker for rice blast resistance gene Pi2. *J. South China Agric. Univ.* **2023**, *44*, 725–734.
18. Jian, P.; Youlun, X.; Jianghui, Y.; Yangjun, Z.; Lin, J.; Xiaomei, T.; Jun, L.; Xiaoping, Z.; Cheng, Y.; Jia, L. Codominance functional marker of bacterial blight resistant xa7 in rice. *Fujian J. Agric. Sci.* **2023**, *38*, 444–453.
19. GB/T 17891-2017; High Quality Paddy. General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China. China Standards Press: Beijing, China, 2018.

20. Dormatey, R.; Sun, C.; Ali, K.; Coulter, J.A.; Bi, Z.; Bai, J. Gene pyramiding for sustainable crop improvement against biotic and abiotic stresses. *Agronomy* **2020**, *10*, 1255. [[CrossRef](#)]
21. Fukuoka, S.; Saka, N.; Mizukami, Y.; Koga, H.; Yamanouchi, U.; Yoshioka, Y.; Hayashi, N.; Ebana, K.; Mizobuchi, R.; Yano, M. Gene pyramiding enhances durable blast disease resistance in rice. *Sci. Rep.* **2015**, *5*, 7773. [[CrossRef](#)] [[PubMed](#)]
22. Chukwu, S.C.; Rafii, M.Y.; Ramlee, S.I.; Ismail, S.I.; Oladosu, Y.; Okporie, E.; Onyishi, G.; Utobo, E.; Ekwu, L.; Swaray, S. Marker-assisted selection and gene pyramiding for resistance to bacterial leaf blight disease of rice (*Oryza sativa* L.). *Biotechnol. Biotechnol. Equip.* **2019**, *33*, 440–455. [[CrossRef](#)]
23. Qu, S.; Liu, G.; Zhou, B.; Bellizzi, M.; Zeng, L.; Dai, L.; Han, B.; Wang, G. The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site–leucine-rich repeat protein and is a member of a multigene family in rice. *Genetics* **2006**, *172*, 1901–1914. [[CrossRef](#)] [[PubMed](#)]
24. Deng, Y.; Zhai, K.; Xie, Z.; Yang, D.; Zhu, X.; Liu, J.; Wang, X.; Qin, P.; Yang, Y.; Zhang, G. Epigenetic regulation of antagonistic receptors confers rice blast resistance with yield balance. *Science* **2017**, *355*, 962–965. [[CrossRef](#)] [[PubMed](#)]
25. Ning, Y.; Liu, W.; Wang, G. Balancing immunity and yield in crop plants. *Trends Plant Sci.* **2017**, *22*, 1069–1079. [[CrossRef](#)] [[PubMed](#)]
26. Karasov, T.L.; Chae, E.; Herman, J.J.; Bergelson, J. Mechanisms to mitigate the trade-off between growth and defense. *Plant Cell* **2017**, *29*, 666–680. [[CrossRef](#)] [[PubMed](#)]
27. Li, W.; Deng, Y.; Ning, Y.; He, Z.; Wang, G. Exploiting broad-spectrum disease resistance in crops: From molecular dissection to breeding. *Annu. Rev. Plant Biol.* **2020**, *71*, 575–603. [[CrossRef](#)] [[PubMed](#)]
28. Oliva, R.; Ji, C.; Atienza-Grande, G.; Huguet-Tapia, J.C.; Perez-Quintero, A.; Li, T.; Eom, J.; Li, C.; Nguyen, H.; Liu, B. Broad-spectrum resistance to bacterial blight in rice using genome editing. *Nat. Biotechnol.* **2019**, *37*, 1344–1350. [[CrossRef](#)] [[PubMed](#)]
29. Cobb, J.N.; Biswas, P.S.; Platten, J.D. Back to the future: Revisiting MAS as a tool for modern plant breeding. *Theor. Appl. Genet.* **2019**, *132*, 647–667. [[CrossRef](#)] [[PubMed](#)]
30. Rasheed, A.; Hao, Y.; Xia, X.; Khan, A.; Xu, Y.; Varshney, R.K.; He, Z. Crop breeding chips and genotyping platforms: Progress, challenges, and perspectives. *Mol. Plant.* **2017**, *10*, 1047–1064. [[CrossRef](#)] [[PubMed](#)]
31. Xu, Y.; Li, P.; Zou, C.; Lu, Y.; Xie, C.; Zhang, X.; Prasanna, B.M.; Olsen, M.S. Enhancing genetic gain in the era of molecular breeding. *J. Exp. Bot.* **2017**, *68*, 2641–2666. [[CrossRef](#)] [[PubMed](#)]
32. Ashkani, S.; Rafii, M.Y.; Shabanimofrad, M.; Miah, G.; Sahebi, M.; Azizi, P.; Tanweer, F.A.; Akhtar, M.S.; Nasehi, A. Molecular breeding strategy and challenges towards improvement of blast disease resistance in rice crop. *Front. Plant Sci.* **2015**, *6*, 886. [[CrossRef](#)] [[PubMed](#)]
33. Jiang, N.; Yan, J.; Liang, Y.; Shi, Y.; He, Z.; Wu, Y.; Zeng, Q.; Liu, X.; Peng, J. Resistance genes and their interactions with bacterial blight/leaf streak pathogens (*Xanthomonas oryzae*) in rice (*Oryza sativa* L.)—An updated review. *Rice* **2020**, *13*, 3. [[CrossRef](#)] [[PubMed](#)]
34. Wu, Y.; Xiao, N.; Yu, L.; Pan, C.; Li, Y.; Zhang, X.; Liu, G.; Dai, Z.; Pan, X.; Li, A. Combination patterns of major *R* genes determine the level of resistance to the *M. oryzae* in rice (*Oryza sativa* L.). *PLoS ONE* **2015**, *10*, e0126130. [[CrossRef](#)] [[PubMed](#)]
35. Varshney, R.K.; Terauchi, R.; McCouch, S.R. Harvesting the promising fruits of genomics: Applying genome sequencing technologies to crop breeding. *PLoS Biol.* **2014**, *12*, e1001883. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.