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Marker-assisted breeding of the elite Rice Restorer Line R202 for resistance to rice bacterial blight and blast diseases, brown plant hopper pest and inhibiting herbicides

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Abstract

Background

Hybrid rice plays a pivotal role in ensuring global food security, yet the weak multiple resistance of restorer lines remains a major bottleneck limiting the stability and environmental sustainability of hybrid varieties. The elite restorer line R202 possesses desirable combining ability and good grain quality, but it is chronically susceptible to bacterial blight, blast, brown planthopper (BPH), and herbicides. Single-trait improvements have proven insufficient to meet the demands of modern intensive rice production.

Results

In this study, we employed backcross breeding combined with marker-assisted selection to introgress the broad-spectrum bacterial blight resistance gene *Xa23*, the blast resistance gene *Pigm*, the brown plant hopper resistance (BPH) gene *Bph6*, and the acetolactate synthase (ALS) inhibiting herbicide resistance gene ALS^{W548M} into the R202 genetic background. After BC_4F_2 generations coupled with stringent foreground selection, we successfully developed the novel restorer line R4202. The hybrid combination 1892S/R4202, derived from R4202, was evaluated under artificial inoculation and (or) natural field infection conditions. Results demonstrated that R4202 and its hybrid exhibited high resistance to both bacterial blight and blast, moderate-to-high resistance to BPH, and good tolerance to ALS-inhibiting herbicides. Multi-location field yield trials showed that R4202 and its hybrid achieved synergistic stacking of these multiple resistances without incurring any significant yield and grain quality penalty; grain yields remained comparable to those of the recurrent parent R202 and its hybrid (with no statistically significant difference, $0.214 < P \text{ value} < 0.910$).

Conclusion

Collectively, R4202 is a novel restorer line that integrates resistances to bacterial blight, blast, BPH, and ALS-inhibiting herbicides, while simultaneously retaining good eating quality and high yield potential. This germplasm provides valuable genetic resources for breeding green, low-input, high-quality hybrid rice varieties

Intruduction

The successful adoption of hybrid rice has greatly contributed to global food security (Cheng et al. 2007). However, with continued population growth and evolving consumer demands, global rice production must increase substantially by 2050 (Yuan et al. 2022). At the same time, cultivation faces mounting biotic challenges, including epidemic diseases such as rice blast and bacterial blight (Fiyaz et al. 2022; Jun et al. 2025; Kaushik et al. 2023), infestations of brown planthopper (BPH) (Sriram et al. 2024), and increasing weed pressure driven by the shift from transplanting to direct-seeding systems due to rising labor costs(Chen et al. 2021). Consequently, developing new rice varieties that integrate multiple resistances to diseases, pests, and herbicides, while maintaining high quality and yield potential, is critical for sustainable food security.

Bacterial blight and rice blast are major diseases that can cause yield losses of 20–50% in severe cases (Fiyaz et al. 2022; Jun et al. 2025; Kaushik et al. 2023). Genetic improvement through resistance (R) gene introgression is one of the most economical and effective strategies for disease management. To date, several crucial R genes have been cloned. Among them, *Xa23* confers exceptionally broad-spectrum and high-level resistance against nearly all known

races of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), and its cognate avirulence gene *avrXa23* is widely distributed among *Xoo* populations, making *Xa23* a highly valuable breeding resource(Wang et al. 2014; Wang et al. 2015). Similarly, *Pigm* provides durable, broad-spectrum resistance to *Magnaporthe oryzae*, the causal agent of rice blast. Notably, *Pigm* exhibits an even broader resistance spectrum than the well-known genes *Pi1*, *Pi2*, and *Pi3*, conferring high resistance or immunity to 29 out of 30 highly virulent strains collected from diverse geographic regions(Deng et al. 2006). Unlike many R genes that are rapidly overcome by pathogen evolution, *Pigm* maintains durable field efficacy through a unique mechanism involving a linked negative regulator that balances resistance with yield stability(Deng et al. 2017; Feng et al. 2022; Xie et al. 2025; Yang et al. 2020).

In recent years, BPH (*Nilaparvata lugens* Distant) has caused devastating losses in China, Japan, Korea, and Vietnam(Bottrell and Schoenly 2012). In 2005 and 2008, BPH-related damage alone resulted in a combined yield loss of 2.7 million tons in China, while an additional 0.4 million tons were lost in Vietnam due to rice ragged stunt virus (RRSV) and rice grassy stunt virus (RGSV), both transmitted by BPH (Bottrell and Schoenly 2012). To date, more than 45 BPH resistance genes have been identified in rice, with a subset—including *Bph1*, *Bph3*, *Bph6*, *Bph7*, *Bph9*, *Bph10*, *Bph14*, *Bph15*, *Bph18*, *Bph21*, *Bph26/2*, *bph29*, *Bph32*, *Bph37*, and *Bph30/Bph40*—having been cloned (Ishwarya et al. 2025). Among them, *Bph6* encodes a protein that localizes to exocysts and interacts with the exocyst subunit OsEXO70E1. Its expression enhances exocytosis and participates in cell wall maintenance and reinforcement. Importantly, *Bph6* activates a coordinated cytokinin, salicylic acid, and jasmonic acid signaling pathway, conferring broad resistance to all four BPH biotypes through prolonged antixenotic and antibiotic effects (Guo et al. 2018; Qiu et al. 2010). Recent evidence indicates that Bph6 interacts with OsEXO70H3 to promote secretion of OsSMASL, thereby regulating lignin accumulation in the cell wall to confer insect resistance(Wu et al. 2022).

Weedy rice is a highly destructive and difficult-to-control weed in paddy fields, particularly in direct-seeding systems, where it can cause yield losses exceeding 40% (Burgos et al. 2014; Sudianto et al. 2013). In China, the shift from transplanting to direct seeding, driven by rising labor costs, has exacerbated weedy rice infestations, affecting over 3 million hectares(CHEN et al. 2020; Jin et al. 2022). Because weedy rice is conspecific with cultivated rice, no conventional herbicide can selectively control it without damaging the crop. Genetic modification has enabled the development of herbicide-resistant rice carrying acetolactate synthase (ALS) mutations, such as the *S627N* allele, which confers resistance to imidazolinone herbicides. However, *S627N* rice has limited field safety and a narrow herbicide spectrum. To overcome these drawbacks, EMS mutagenesis was performed on the elite indica cultivar Huang Hua Zhan, and a novel mutation ALS^{W548M} was identified(Chen et al. 2021). Compared with *S627N*, this mutant confers broad-spectrum resistance to multiple ALS-inhibiting herbicides, substantially higher resistance levels, and no yield penalty, making it a valuable resource for weed management in rice(Chen et al. 2021).

While pyramiding one to three resistance types is common in rice breeding programs (Chen et al. 2025; Li et al. 2025; Wang et al. 2017; Wu et al. 2026), the simultaneous stacking of resistance to bacterial blight, blast, BPH, and ALS-inhibiting herbicides in a single restorer line is rarely reported. In this study, we aimed to introgress *Xa23*, *Pigm*, *Bph6*, and ALS^{W548M} into the genetic background of the elite restorer line R202 using marker-assisted backcrossing. Through successive crossing, backcrossing, and self-pollination with rigorous foreground selection, we developed a all four homozygous resistance genes line, designated R4202. We systematically evaluated its agronomic traits, resistance spectra, and hybrid performance. The development of R4202 provides a novel restorer line integrating multiple resistances without yield or grain-quality penalties, offering significant practical value for green hybrid rice production.

Materials and Methods

Rice materials

The recurrent parent of this study was the widely used restorer line R202. The *Xa23* donor parent was line CBB23(*Xa23*), which carry *Xa23* in Jingang 30 (JG30) background(Wang et al. 2014). The blast resistance gene *Pigm* and brown plant hopper resistance gene *Bph6* donor line was Huahui 3006 (R3006) (Deng et al. 2024). The herbicide ALS^{W548M} gene donor was rice JTD001(Chen et al. 2021). The widely applied thermosensitive genic male sterile line 1892S used as a parent for hybrid rice(Yanchang et al. 2021).

Marker-assisted selection

The molecular markers used in this study are listed in Table 1. PCR amplification was performed on an Applied Biosystems Veriti 96-well thermal cycler. The reaction system (15 μL) contained: 10 ng genomic DNA, 0.15 mM dNTPs, 0.15 mM primers, 2 μL 10× PCR buffer, and 1 U Taq DNA polymerase. The reaction program was: 94°C for 3 min; then 33 cycles of 94°C for 20 s, annealing temperature 55 for 25, and extension at 72°C for 0.5-1 min depending on product length; followed by a final extension at 72°C for 5 min. All amplification products were detected by electrophoresis on 3.0% in 1× TAE buffer.

Table 1
Molecular markers and their characteristics used in this study

Target gene	Marker name	Type and size of marker	Primer sequence (5'-3')	Reference
Xa23	M-Xa23	Co-dominant	F: TTGCTCAAGGCTAGGAAAATG R: -CCCCATCAACGAACTACAGG	23
Pigm	Pigm-194bp	Co-dominant STS 194bp	F: CCGAGGCAAAGTTCTAACTG R: CTAAGAGCGACGACGATAGG	Designed according to sequence reference(Deng et al. 2006; Qu et al. 2006)
Bph6	Bph6-291	Dominant STS 291bp	F:CGACGGTATATAGTGAAGTTG R:GTGACTCAGCCTTGTGTTTC	Designed according to sequence reference(Guo et al. 2018)
bph6	bph6-217	Dominant STS 217bp	F:CATGCAGCAACAAGACCTTAT R:TGACTCAGCCTTCCCCTGCA	Designed according to sequence reference(Guo et al. 2018)
ALS	ALSM208	Dominant STS208bp	F: CTGGAGTCTCGAGCATCTTC R:TTGGGTATGGTTGTGCAAAT	Designed according to sequence reference(Chen et al. 2021)
als	alsw300	Dominant STS 300 bp	F:ATCGCTACTGGTGTTGGGCA R:CTTGTAAAACCTATCCTCCCA	Designed according to sequence reference(Chen et al. 2021)

Backcross breeding procedure

R202 was used as the recurrent parent. Donor parents were JTD001 for ALS^{W548M}, R3006 for *Pigm* and Bph6, CBB23(*Xa23*) for *Xa23*. After four backcrosses, BC_4F_1 plants carrying each target gene were obtained. To pyramid the four genes, a double cross was made among these BC_4F_1 plants BC_4F_1(*Xa23*), BC_4F_1(*Pigm*,*Bph6*), and BC_4F_1(*ALSM*)). The double-cross F_1 plants, heterozygous for all four genes, were self-pollinated to produce subsequent generations. Marker-assisted selection was used to track and select the target genes. Finally, a plant homozygous for all four target genes was identified in a BC_4F_2 (or later) population and designated R4202 (Fig. 1).

Phenotypic evaluation

Evaluation of *ALS* inherbiting herbicide Imazapic

Dry rice seeds were soaked for 24 hours in a 30 mg/L solution of imazapic (an *ALS*-inhibiting herbicide) and then washed once before germination. The germinated seeds were cultured in hydroponics on January 28, 2026, or sown in the field on May 12, 2026. Photographs were taken on February 5, 2026, or May 21, 2026, respectively. Evaluation standards was whether most of seedling survived or died.

Evaluation of Brown plant hopper resistance

Seeds of the tested rice varieties and the susceptible control variety, after germination, were sown in plastic pots (60 cm × 40 cm × 10 cm). Each row was 20 cm long with a row spacing of 5 cm, and 20 seeds were sown per row. Three replications were set for both the susceptible TN5 and resistant control variety R3006, and three replications for each tested variety. After seed germination and seedling emergence, seedlings were thinned to 15–20 plants per row. When the seedlings reached the 2–3 leaf stage, 5–7 1st–2nd instar nymphs of planthopper were introduced per plant on average. When the mortality rate of the susceptible control variety TN1 reached approximately 95%, the resistance level was evaluated based on the mortality rate (Table 1).

Table 2
Evaluation standard for planthopper resistance in rice based on seedling mortality

Resistance grade	Seedling mortality%	Resistance description
0	<1.0	Immunity
1	1.1–10.0	Highly resistant
3	10.1–30.0	Resistant
5	30.1–50.0	Moderately resistant
7	50.1–70.0	Moderately susceptible
9	>70.1	Susceptible
Rice blast resistance		

Two dominant *M. oryzae* isolates, ZB13 and 11-3-1-1-2, widely distributed in China, were used for inoculation experiments in Hefei, China, during June-July 2026. Both isolates were collected from Anhui, China, and were cultured on prune agar medium (40 ml prune juice, 20 g/L agar, 5 207 g/L sucrose, 5 g/L lactose, 1 g/L yeast extract, pH 6.5). Rice plants at the 4-leaf stage were inoculated with a spore suspension at a concentration of 1×10^5 spores/ml. Immediately after inoculation, the plants were placed in a dark box for 24 hours at approximately 25°C and 90% relative humidity. Following this incubation period, the plants were transferred to a growth chamber and maintained at 25–28°C under a 12/12-hour (light/dark) photoperiod and 90% relative humidity. Disease evaluation was conducted 7 days after inoculation.

Bacterial blight resistance

Resistance to bacterial blight was evaluated using the leaf-clipping method as described(Gu et al. 2004). *Xoo* strains were cultured on PSA medium at 28°C for 2 days, then suspended in sterile water to OD_{600} = 0.5 for inoculation. Lesion length was measured 14 days after inoculation. The disease symptoms were scored as resistant (R, LL < 3.0 cm),

moderately resistant (MR, 3.0 cm < LL < 6.0 cm), moderately susceptible (MS, 6.0 cm < LL < 9.0 cm) or susceptible (S, LL > 9.0 cm).

Agronomic traits and rice quality evaluation

Field trials

Two field trials were conducted using rice varieties R202, R4202, and their hybrid. Field trial 1 and field trial 2 were carried out in Hefei (Anhui, China) during the summer seasons (May–October) of 2024 and 2025, respectively. Germinated seeds were sown on May 1 and transplanted on June 1. In each trial, paired parental lines and the hybrid were planted in adjacent plots, each plot measuring approximately 10 m², with a plant spacing of 16.7 cm × 26.7 cm. Three replicate plots were planted for each parental line or hybrid line.

Total nitrogen application was 180 kg/ha, with an N:P:K ratio of 1:0.5:0.9. Basal fertilizer accounted for 50% of total N; tillering fertilizer and panicle fertilizer each accounted for 25%, applied 7 days after transplanting and around stem elongation, respectively. All phosphate fertilizer was applied as basal. For potassium, 60% was applied as basal and 40% as panicle fertilizer.

The key agronomic traits of rice, including growth duration, plant height, effective panicle number per plant, total grains per panicle,seed setting rate, 1000-grain weight, and yield, were measured from 10 plants per plot, giving a total of 30 plants per variety across the three plots. P-values for each trait were calculated using Microsoft Office Excel 2021 based on a two-tailed paired-sample t-test.

Rice quality analysis

Harvested grains were evaluated for grain length, length/width ratio, degree of chalkiness, amylose content, gel consistency, alkali spreading value according to NY/T 593–2021(China 2021). Grading standards followed the same reference (China 2021)

Results

Breeding of the high-quality, multi-resistant line R4202

To pyramid the *ALSM*, *Bph6*, *Pigm*, and *Xa23* genes in the R202 genetic background, we first generated backcross lines carrying each or two genes by marker-assisted selection, using R202 as the recurrent female parent and JTD001(*ALSM*), R3006 (*Bph6*, *Pigm*), and CBB23(*Xa23*) as donor lines (Fig. 1). Theoretically, after 4 times backcrosses, the BC_4F_1 plants each possessed approximately 96.9% of the genetic material of the recurrent parent R202. The morphological phenotypes of the BC_4F_1(*ALSM*), BC_4F_1(*Bph6*,*Pigm*), and BC_4F_1(*Xa23*) plants were similar to that of R202. To combined *ALSM*,*Bph6* and *Pigm*, The F1 Plant ABP(*ALS,Bph6,Pigm*) was produced from the cross between B4F1(*ALSM*) and B4F1*(Bph6,Pigm*). Likewise, plant BPX (*Bph6,Pigm,Xa23*) was obtained from from F1 progeny of B4F1(*Bph6,Pigm*) × B4F1(*Xa23*)(Fig. 1). ABP (*ALS,Bph6,Pigm*) was then crossed with BPX (*Bph6,Pigm,Xa23*) to pyramid the four genes. Ninety-six plants were obtained from the cross and three of them, designated F_1(*ALSM, Bph6, Pigm, Xa23*), contained heterozygous alleles at all four target loci (Fig. 1). The three F_1(*ALSM, Bph6, Pigm,Xa23*) plants were self-pollinated to obtain as much F_2 seed as possible. The F_2 seeds from these three plants were used to generate two F_2 populations, which together contained 768 F_2 plants. The genotype at each locus was determined using a specific molecular markers *ALSM-208*/alsw300, Bph6-291/bph6-217, Pigm-194 and MXa23, respectively (Table 1). One of F2

plants contained homozygous alleles at all of the four loci for selection. The plant (line 1) designated as R4202 (genotype: *ALSM/ALSM, Bph6/Bph6, Pigm/Pigm, Xa23/Xa23*) was selected for further study(Fig. 2)

Line 1, carrying homozygousalleles at all of the four loci, were identified from the F2 population derived from the double cross among BC_4F_1(*ALSM*), BC_4F_1(*Bph6,Pigm*), and BC_4F_1(*Xa23*). The F2 plant line 1 was selected and designed as R4202.

R4202 and its hybrid 1892S/R4202 are resistant to ALS inhibiting herbicide imazapic

To evaluate $ALSW^{548M}$ inhibiting herbicide imazapic resistance, Dry rice seeds were soaked on Janary 28,2026 and May 12,2026 respectively for 24 hours in a 30 mg/L solution of imazapic (an ALS-inhibiting herbicide) and then washed once before germination. Both resistant and susceptible seeds germinated, but susceptible seedlings rarely developed roots. After germination, a clear difference was observed between the resistant and susceptible phenotypes (Fig. 3A and Fig. 3B). Resistant seeds of R4202,JTD001 and 1892S/R4202 developed into normal seedlings with roots, stems, and leaves(almost seedling survived), whereas susceptible seeds of R202,1892S/R202 failed to establish normal seedlings (almost all seedlings died) (Fig. 3A and Fig. 3B). These results indicate that R4202 and its hybrid 1892S/R4202, carrying the ALS^{W546M} gene, are resistant to *ALS* inhibiting herbicide imazapic. The R4202 is also are resistant to *ALS* inhibiting herbicide imazapic at tillering stage while R202 are susceptible to it (Fig. 3C)

R4202 and its hybrid show resistance to brown plant hopper

The resistance of TN5, R202, R4202, 1892S, 1892S/R202, and 1892S/R4202 to was evaluated. R3006 was as resistance control while TN5 as susptible control. R4202 was resistant and 1892S/R4202 was moderately resistance while 1892S, R202 and 1892S was susceptible to brown plant hopper (Table 3, Fig. 4A). These results demonstrate that brown hopper resistance genes (*Bph6*) present in R4202 and its hybrid provide resistance (homozygote) or moderately resistanc(heterozygote) to BPH.

In F1 heterosis and hybrid rice seeds purity indentification field under natral condition in 2025 spring season, 1892S/R4202 showed high resistance to brown plant hopper, while 1892S/R202 suseptible to plant hopper (Fig. 4B). The result further verified the *Bph6* gene in hybrid rice provide resistance to BPH.

Table 3
Evaluation for brown plant hopper in R4202, R202 and their Hybrids

Variety	Grade of resistance
R3006	1, High Resistance
R202	7, Seceptible
R4202	1, High Resistance
1892S	7, Suceptible
1892S/R202	7, Suceptible
1892S/R4202	5, Moderately Resistance
TN5	9, High suceptible

R4202 and its hybrid 1892S/R4202 are resistant to bacterial blight and rice blast

The resistance of CBB23(*Xa23*), R202, R4202, 1892S, 1892S/R202, and 1892S/R4202 to seven *Xoo* strains from China was evaluated. R202 was susceptible or moderately susceptible to all five tested *Xoo* strains, while CBB23(*Xa23*) was resistant. The improved line R4202 was resistant to all strains (Fig. 5A-5E). 1892S was susceptible or moderately susceptible to the five *Xoo* strains, and the control hybrid 1892S/R202 was also susceptible (Fig. 5A-5E). The hybrid 1892S/R4202 showed high resistance to all five tested *Xoo* strains, similar to its male parent R4202 (Fig. 5A-5E). These results demonstrate that the bacterial blight resistance genes (*Xa23*) present in R4202 and its hybrid provide broad-spectrum and highly effective resistance to multiple *Xoo* strains.

To assess disease resistance to rice blast, 4-leaf seedlings of R3006, R202, R4202, 1892S, 1892S/R202, and 1892S/R4202 were challenged with two dominant *Magnaporthe oryzae* isolates, ZB13 and 11-3-1-1-2, collected from Anhui, China. The experiments were conducted in June-July 2026. R3006 was resistant to both isolates. R202, the female parent 1892S, and its hybrid 1892S/0M052 were completely susceptible to both isolates (Figs. 6A, 6B). No blast lesions were observed on R4202 or its hybrid 1892S/R4202 (Figs. 6A, 6B). These results demonstrate that R4202, carrying the *Pigm* gene, and its hybrid 1892S/R4202 confer resistance to both *M. oryzae* isolates ZB13 and 11-3-1-1-2 in China. Field experiment under natural condition further conformed this results(Fig. 6C).

R4202 and its hybrid show agronomic traits similar to R202 and its hybrid

The performance of R4202, R202, and their hybrid combinations (1892S/R202 and 1892S/R4202) under two-year field conditions is shown in Table 3. R4202 and R202, as well as 1892S/R4202 and 1892S/R202, had similar growth duration, plant height, number of effective panicles per plant, panicle length, total grains per panicle, seed setting rate, and 1000-grain weight (Table 3). Yield differences between R4202 and R202, and between 1892S/R4202 and 1892S/R202, were not significant (Table 3). In summary, the main agronomic traits of R4202 and its hybrid 1892S/R4202 were similar to those of R202 and the control hybrid, respectively. This indicates that the introgression of *Xa23*, *Pigm*, *ALSM* and *Bph6* into R4202 or its hybrid line (1892S/R4202) did not significantly affect their main agronomic traits.

Table 3
Evaluation of main agronomic traits of R202, R4202 and hybrid rice lines

Field trial	varieties	Growth period (day)	plant height(cm)	Effective panicles (10K/hm^2.)	Grain number per panicle	Seed setting rate(%)	thousand-grain weight(g)	Yields(t/hm^2)
1	R202	127	116.7 ± 0.5	246.3 ± 8.8	208.7 ± 8.3	83.1 ± 0.5	18.1 ± 0.0	7.7 ± 0.1
	R4202	126	115.7 ± 0.2	260 ± 17.7	200.3 ± 9.5	78.3 ± 0.7	18.6 ± 0.4	7.6 ± 0.2
	T-test (P-value)	-	1.0	0.259	0.331	0.274	0.750	0.214
	1892S/R202	137	127.3 ± 1.5	6.9 ± 0.5	167.2 ± 10.5	87.7 ± 0.4	22.6 ± 0.6	10.8 ± 0.4
	1892S/R4202	136	127.9 ± 1.2	6.7 ± 0.5	160.5 ± 0.8	85.9 ± 3.4	23.2 ± 1	10.3 ± 0.4
	T-test (P-value)	-	0.749	0.659	0.412	0.426	0.239	0.365
2	R202	128	116.7 ± 1.7	318.8 ± 62.5	187.9 ± 17.0	86 ± 2.6	18.2 ± 0.5	9.2 ± 1.2
	R4202	127	119.7 ± 2.9	270.3 ± 13.1	186.3 ± 23.0	83.4 ± 1.2	18.8 ± 0.3	8.7 ± 0.6
	T-test (P-value)	-	0.189	0.402	0.794	0.132	0.350	0.296
	1892S/R202	138	123.2 ± 1.4	6.8 ± 0.2	168.6 ± 8	83.6 ± 2.6	22.2 ± 1.4	10.1 ± 0.2
	1892S/R4202	137	125.7 ± 3.3	7.8 ± 1.1	176.2 ± 3.5	82.9 ± 0.8	22.9 ± 0.3	10.0 ± 1.3
	T-test (P-value)	-	0.208	0.234	0.349	0.592	0.391	0.910

Note: T-test indicates no significance for tested traits between R202 and R4202 or between their hybrids($P > 0.05$).

R4202 and its hybrid possess as good grain quality as that of R202 and its hybrid

Grain quality parameters of R4202, R202, and their hybrids (1892S/R4202 and 1892S/R202) were measured. According to length/width ratio, R4202, R202, and their hybrids are medium-grain rice. The degree of chalkiness was grade 1 for all R4202, R202 and their hybrids 1892S/R4202 and 1892S/R202 (Table 4). R4202 and its hybrid 1892S/R4202 showed low amylose content and soft gel consistency similar to R202 and its hybrid 1892S/R202, respectively. R4202, R202, 1892S/R4202, and 1892S/R202 all had high alkali spreading values and low gelatinization temperatures (Table 4). These results demonstrate that the introgression of the genes *Xa23*,*Pigm*, *Bph6* and *ALS* into R202 and its derived two-line hybrid line resulted in good cooking and eating quality similar to R202 and the control hybrid.

Table 4
Grain quality of R202, R4202 and hybrid rice lines

Item	The requirements of standard of grade 1	R202	R4202	1892S/R202	1892S/R4202
Alkali spreading value and Gelatinization temperature	≥ 6.0	7.0	6.5	6.6	6.4
Gel consistency f (mm)	≥ 60	68	73	74	76
Degree of chalkiness d(%)	≤ 1.0	0.0	0.7	0.9	0.8
Amylose content e(%)	13.0 ~ 18.0	16.8	16.8	17.7	16.4
Grain length (mm)	5.6 ~ 6.5	6.4	6.3	6.5	6.6
Ratio of length to width c	/	3.2	3.1	3.3	3.2

Discussion

Marker-assisted selection (MAS) provides an efficient and precise strategy for simultaneously pyramiding multiple target genes, and in this study we successfully introgressed Xa23, Pigm, Bph6, and ALSW548M into the restorer line R202 through four backcross generations coupled with stringent foreground selection. This approach enabled the rapid development of the homozygous line R4202 with a highly recovered recurrent-parent genetic background, substantially improving breeding efficiency compared with conventional phenotypic selection.

A key advantage of MAS in this breeding pipeline was the capacity for early, non-destructive, and parallel selection at the seedling stage. Phenotypic evaluations for blast, bacterial blight, and BPH resistance are not only time-consuming—requiring extensive inoculation, controlled environments, and recovery periods—but also physically damage the plants, often compromising subsequent trait evaluation or seed production. By circumventing these constraints, MAS shortened the breeding cycle by approximately 2–3 generation for homozygotous selection and made the simultaneous pyramiding of multiple resistance genes logistically feasible on a practical scale. Moreover, precise foreground and four times backcrossing selection through MAS minimized linkage drag, avoiding the co-introgression of undesirable agronomic traits (Jin et al. 2022; Luo et al. 2016).

Notably, R4202 and its hybrid combination 1892S/R4202 exhibited no significant differences in key agronomic traits or grain yield compared with the recurrent parent R202 and the control hybrid, confirming that stacking these four resistance genes did not impose a detectable yield penalty. This outcome aligns with previous reports demonstrating that MAS-mediated pyramiding of resistance, stress-tolerance, and quality genes can effectively improve comprehensive performance without compromising productivity(Li et al. 2025; Wu et al. 2026). The absence of yield drag can be attributed to the judicious choice of the component genes. Specifically, *Pigm* is known to carry a linked negative regulator that mitigates pleiotropic effects on yield(Deng et al. 2017; Feng et al. 2022; Xie et al. 2025; Yang et al. 2020), while the ALSM allele was specifically selected for its neutral impact on agronomic performance(Chen et al. 2021). Likewise, *Xa23* and *Bph6* have been widely deployed without major negative effects on yield potential (Guo et al. 2018; Wang et al. 2015) suggesting that their signaling pathways do not impose substantial metabolic costs under normal growing conditions.

The introgression of *ALSM* is particularly significant in the context of rising direct-seeding adoption in China, where weedy rice infestations have become increasingly severe(CHEN et al. 2020; Jin et al. 2022). Field trials across multiple Chinese regions have demonstrated that the W548M mutation confers high-level, broad-spectrum resistance to ALS-

inhibiting herbicides, providing effective weedy rice control without crop injury. Beyond direct-seeding systems, this herbicide resistance also holds practical value in hybrid seed production, including roguing of off-types in seed production fields.

While our results demonstrate the successful development of R4202, several considerations warrant further investigation. The field trials were conducted over a limited number of seasons and locations; multi-year, multi-site evaluations are needed to confirm the stability and durability of the pyramided resistance under diverse environmental conditions. Additionally, although *Pigm* confers durable blast resistance, long-term monitoring of pathogen populations is advisable to detect any potential breakdown of resistance due to emerging virulent strains.

Conclusion

In conclusion, we successfully developed the novel restorer line R4202, which integrates four resistance traits—bacterial blight, blast, BPH, and ALS-inhibiting herbicides—into a single genetic background, while maintaining high yield potential and good grain quality. This line achieves a synergistic improvement of multiple resistance, productivity, and quality, providing valuable germplasm for breeding green, low-input hybrid rice varieties. The R4202 line offers a promising genetic resource for sustainable rice production, particularly in direct-seeding systems where weed pressure and pest/disease outbreaks are intensifying. Future work will focus on evaluating its performance across broader environments and incorporating it into elite hybrid combinations for commercial deployment.

Declarations

Author Statement

Yanchang Luo and Zhixiang Luo designed the experiments. Yanchang Luo conducted the experiments with assistance from Hongyang Du,Xihan Cong, Xinmin Ruan, Fuzhi Shi, and Yuxiang Luo. Yanchang Luo wrote the manuscript. Zhixiang Luo revised the manuscript.

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Author Contribution

Yanchang Luo and Zhixiang Luo designed the experiments. Yanchang Luo conducted the experiments with assistance from Hongyang Du,Xihan Cong, Xinmin Ruan, Fuzhi Shi, and Yuxiang Luo. Yanchang Luo wrote the manuscript.

Zhixiang Luo revised the manuscript.All authors reviewed the manuscript

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Data availability

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Declarations

Competing Interests

The authors declare no competing interests.

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Figures

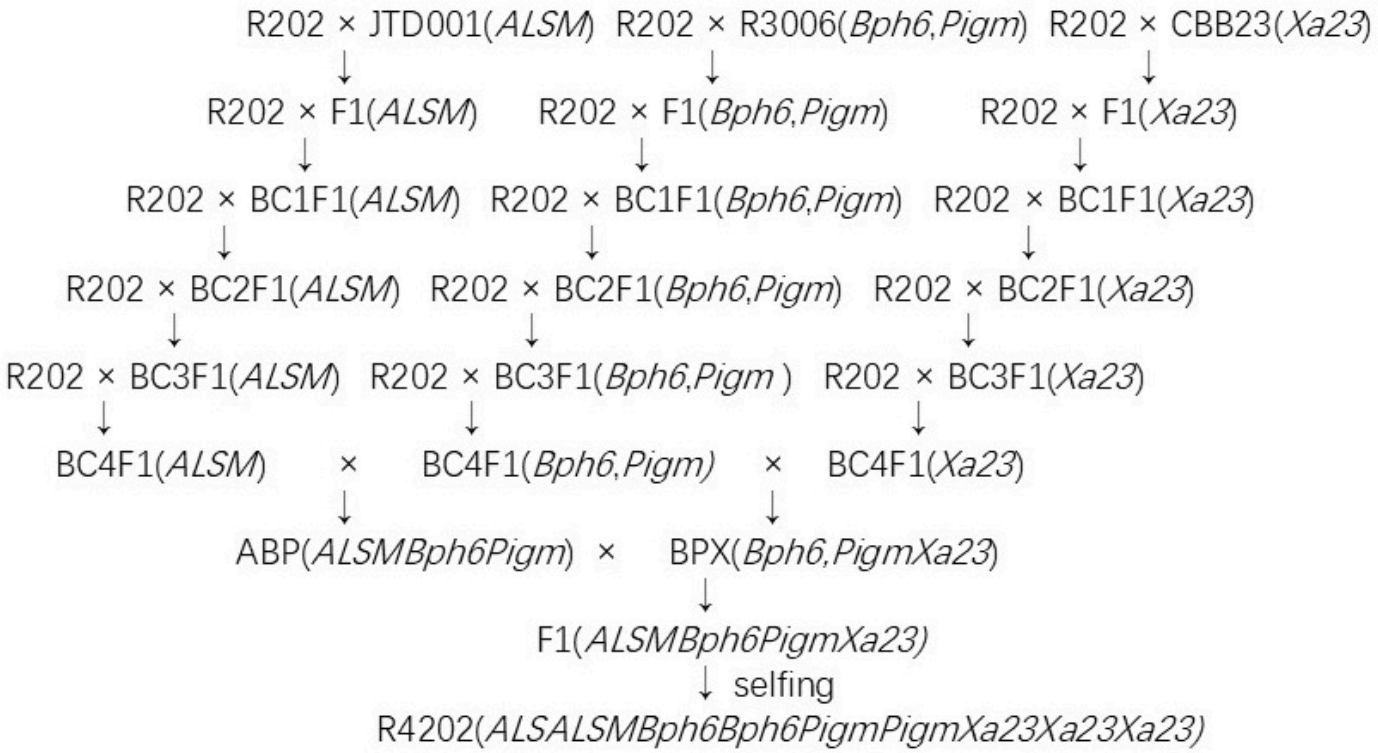


Figure 1

Breeding procedure of marker-assisted selection for *ALSM*, *Bph6*, *Pigm*, and *Xa23* in R4202. ALSM, acetolactate synthase (ALS) inhibiting herbicide mutation ALS^{W548M};*Bph6,*Brown plant hopper resistance gene;*Pigm,* Rice blast resistance gene; *Xa23*, bacterial blight resistance gene

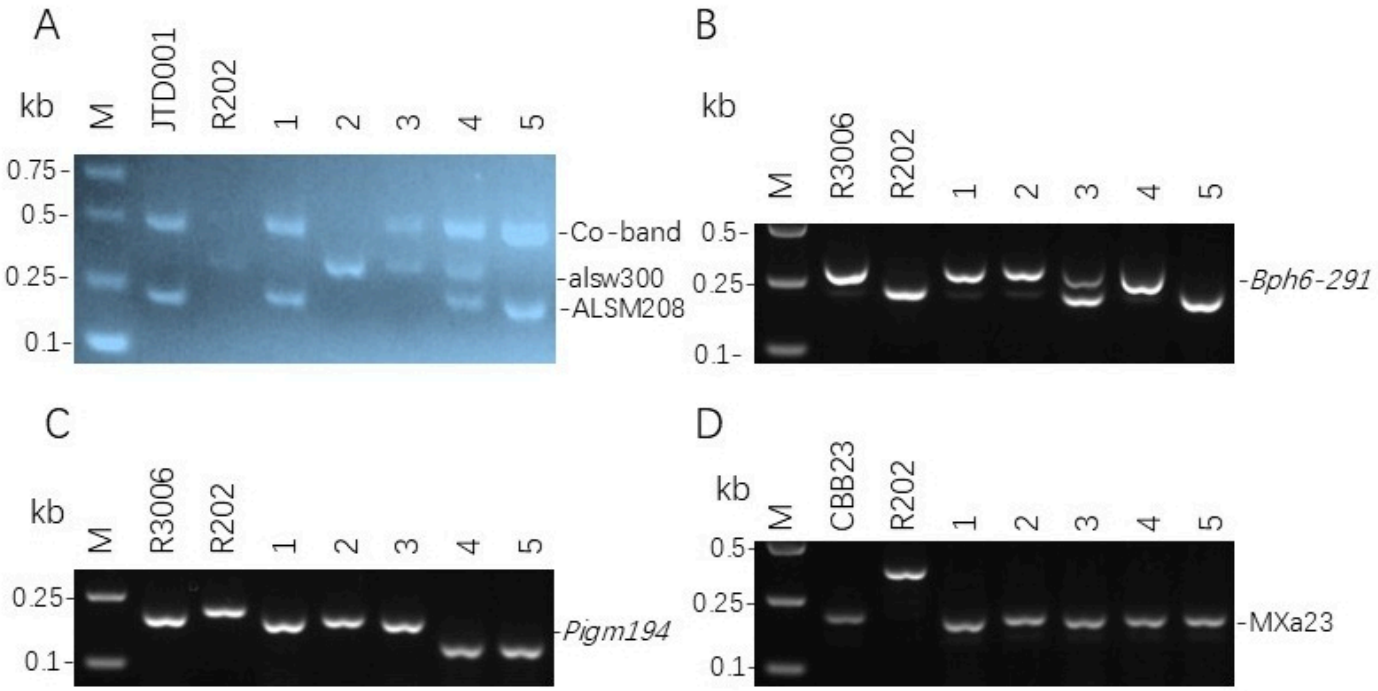


Figure 2

Marker-assisted selection of R4202

Patterns of molecular markers in R4202(line1) ,R202 and donor lines.

A Molecular markers ALSM-208,alsw-300 were designed based on the gene sequence respectively, when the simples was amplified using two dominant makers ALSM-208 and alsw300 together, ALS-208F and alsw-300R formed a common band 470bp

B Bph6-291,bph6-217 was designed based on the genes sequence diferance, when the simples was amplified using two dominant makers

C *Pigm*-194 was designed according to the diferance among *Pigm*, *Pi9* and *Pi2*gene sequence.

D MXa23 was closly linked to *Xa23* gene.

Line 1, carrying homozygousalleles at all of the four loci, were identified from the F2 population derived from the double cross among BC_4F_1(*ALSM*), BC_4F_1(*Bph6,Pigm*), and BC_4F_1(*Xa23*). The F2 plant line 1 was selected and designed as R4202.

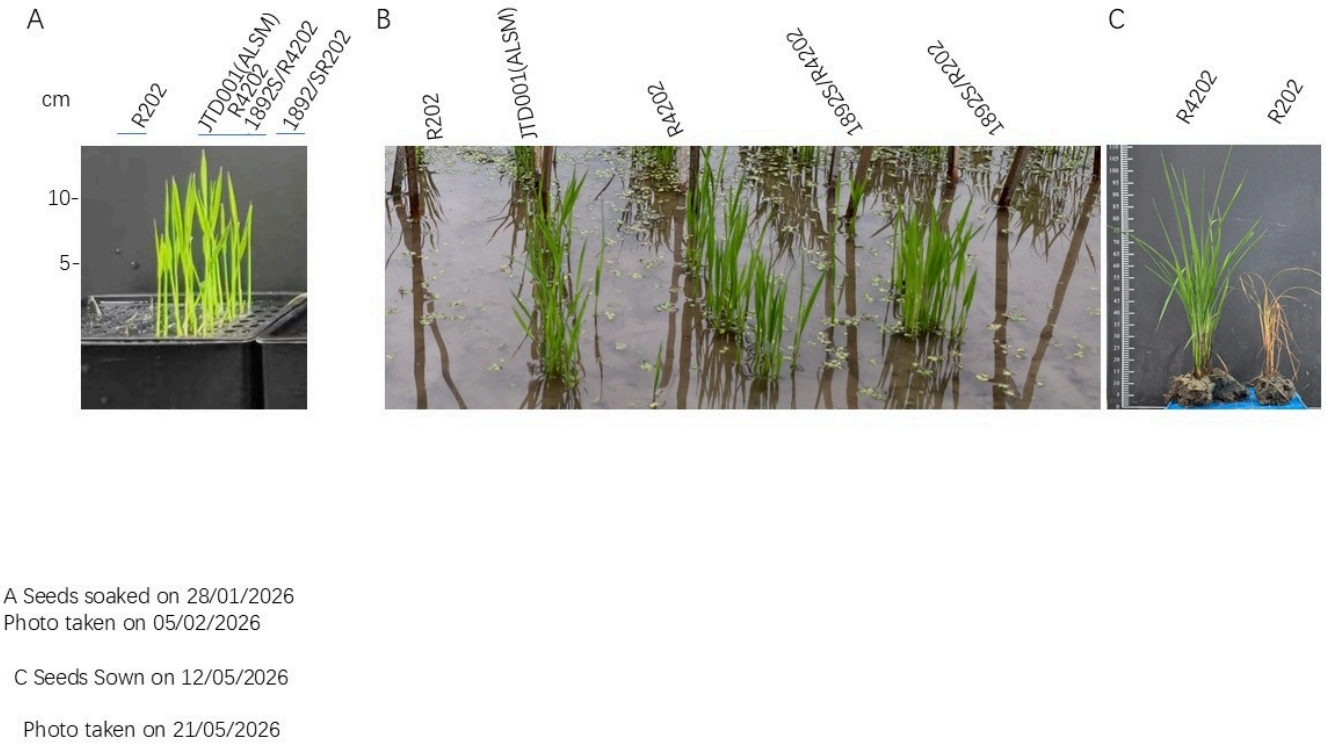


Figure 3

Evaluation for ALS inhibiting herbicide in R4202, ALS donor line, recipient and their Hybrids

A Phenotype of ALS-inhibiting herbicide imazapic treatment to dry seeds then geminated seeds placed in plate culturing in hydroponics

B Phenotype of ALS-inhibiting herbicide imazapic treatment to seeds then geminated seeds sown on seedling field

C Phenotype of ALS-inhibiting herbicide imazapic treatment to seedling at tillering stage. Images were taken 14 days after imazapic treatment (active element containt 0.1%)

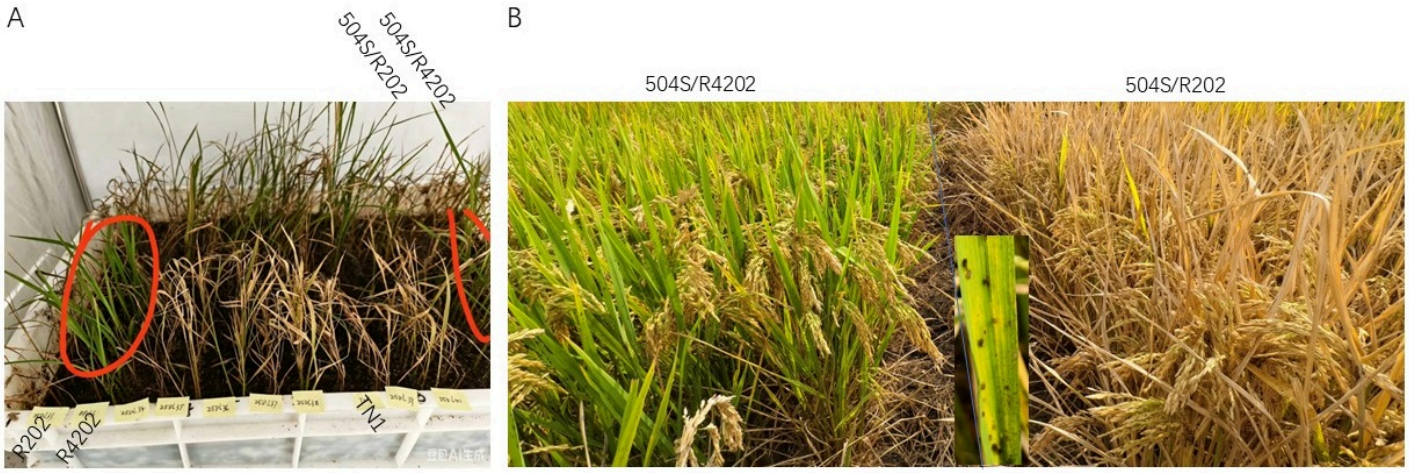


Figure 4

Evaluation for brown plant hopper in R4202, R202 and their Hybrids

A Evaluation for brown plant hopper in R4202 R202 and their hybrids at seedlings with the 2–3 leaf stage

B 1892S/R4202 and 1892S/R202 were grown in Lingshui in 2024-2025 under nature condition. 1892S/R4202(on the left) was resistance to BPH while 1892S/R202(on the right) susceptible to BPH.

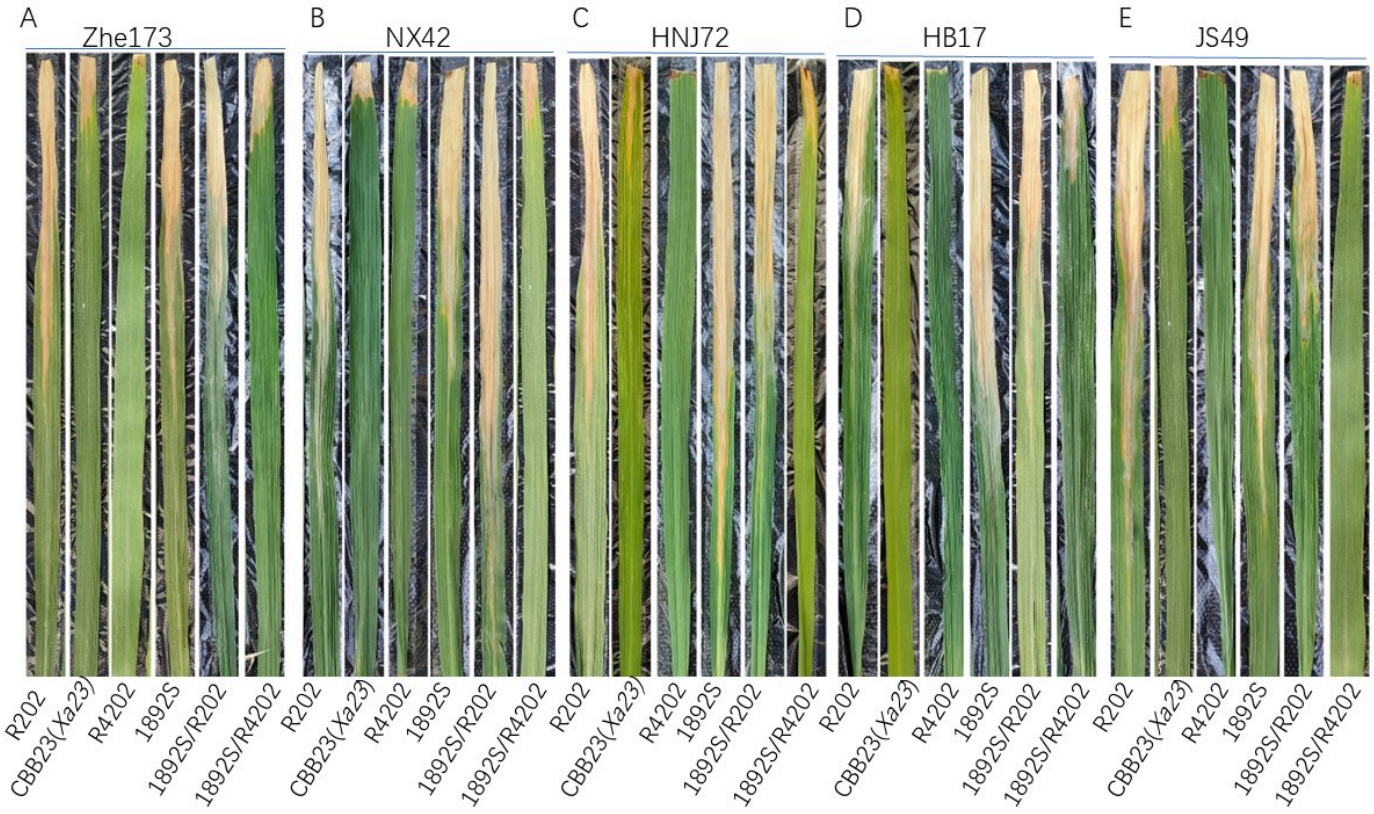


Figure 5

Disease evaluation of R202, R4202, their hybrids and parental lines to rice bacterial blight strains (A, Zhe 173;B,NX42;C,HNJ72; D,HB17; E, JS49)

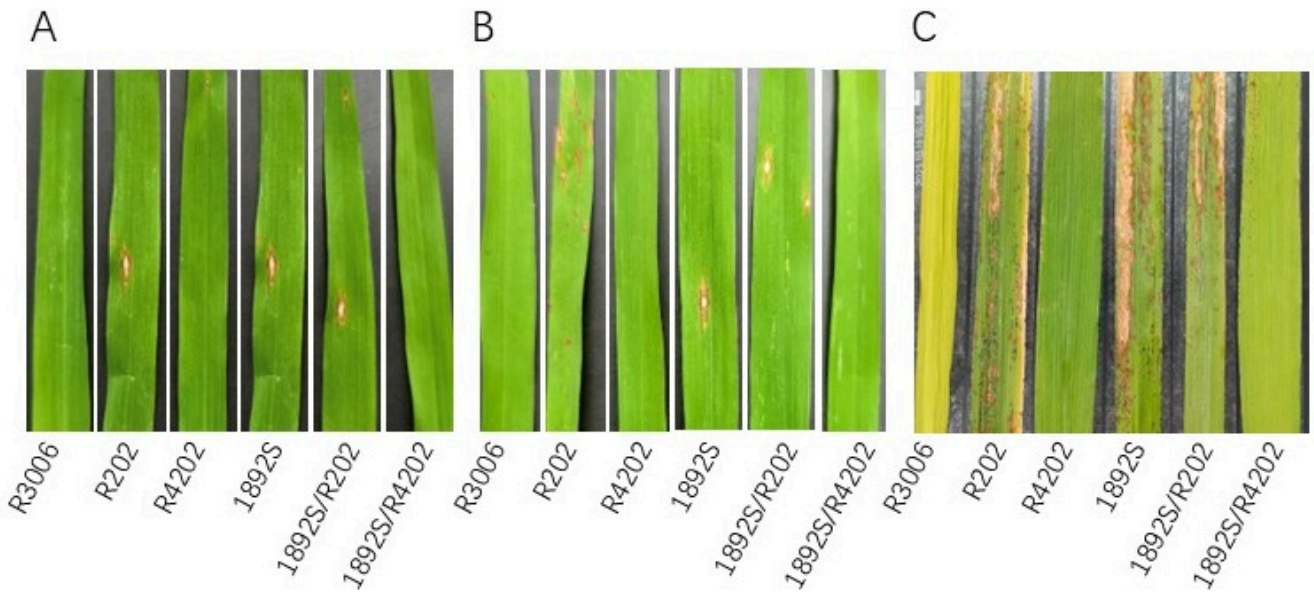


Figure 6

Disease Evaluation for Rice Blast in R4202, Parental Lines, and Their Hybrids

At the 4-leaf stage, rice seedlings of R3006, R202, R4202, 1892S, 1892S/R202, and 1892S/R4202 were inoculated with the dominant *Magnaporthe oryzae* isolates ZB13 (A) and 11-3-1-1-2 (B), collected from Anhui, China. Images were taken 7 days post-inoculation to assess disease progression; leaves of R3006, R202, R4202, 1892S, 1892S/R202, and 1892S/R4202 collected from field under natural condition at later stage in Feixi basement in 2025 (C)