



Enhancing the blast resistance of an elite thermo-sensitive genic male sterile line (TGMS) Longke638S and its derived hybrid varieties by incorporating *Pigm* gene

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Abstract

Rice blast, caused by the fungal pathogen *Magnaporthe oryzae*, is one of the most destructive diseases of rice worldwide. The utilization of host resistance (*R*) genes in rice breeding program is considered as the most economical, effective, environment-friendly strategy for rice blast control. The *R* gene *Pigm*, shows high, broad-spectrum and durable resistance to rice blast. Here, we report the successful integration of *Pigm* into Longke638S (LK638S), an elite thermo-sensitive genic male sterile (TGMS) line in hybrid rice production in China. The integration significantly enhanced the blast resistance of LK638S and the derived hybrid varieties demonstrated exceptional performance in both yield and blast resistance. The improved line Longzhen36S (LZ36S), which recovered 91.84% of the recurrent parent genome. LZ36S exhibited a high blast resistance frequency of 96.4% against 28 blast isolates. Furthermore, the LZ36S-derived hybrids exhibited enhanced resistance to both seedling and panicle blast compared to LK638S-derived hybrids carrying the heterozygous *Pi2* gene, all without yield penalty. A total of ninety LK638S derived hybrid varieties have been state or provincial approved and certified with an annual promoting area exceed 964.0 thousand hectares. The LZ36S-derived hybrids can serve as improved versions with enhanced blast resistance, making them viable replacements for LK638S-derived hybrids in commercial production. Moreover, sixteen LZ36S-derived hybrid varieties, all possessing moderate (MR) or high (R) level blast resistance, along with excellent yield and grain quality, have been state or provincial approved and certificated. These LZ36S-derived hybrids show great potential for rapid commercialization, with promoting area of ~200 thousand hectares by 2023.

Keywords Hybrid rice · Blast resistance · *Pigm* · Thermo-sensitive genic male sterile · Marker-assisted selection

Introduction

Rice (*Oryza sativa* L.) is the staple food for about half of over three billion people, particularly in Asia. Over the past five decades, rice production has been almost doubled due to the introduction of the semi-dwarf gene *sd1*, exploitation of hybrid rice, improvement in cultivation management, and utilization of fertilizers and fungicides. To meet the growing demand of an expanding human population, there is a pressing need for a continuous increase in rice production. However, rice production faces substantial challenges due to constraints in farmland availability, water availability, soil fertility, climate change impacts, as well as pests and diseases (Takeda et al. 2008). In particular, rice blast, caused by the fungus *Magnaporthe oryzae*, is the most destructive disease that severely threatens global rice production. It has been reported that rice blast disease can cause an annual grain yield loss ranging from 10 to 30%, and in some cases, it can even result in a total loss of yield reaching up to 100% during disease epidemics (Skamnioti and Gurr 2009).

Development of resistant cultivars carrying *R* genes has been proved to be the most effective, economic and environmentally-friendly strategy to control rice blast (Zeigler et al. 1994). More than 100 *R* genes and over 350 quantitative trait loci (QTLs) associated with blast resistance have been identified (Tanweer et al. 2015; Wang et al. 2017). Among them, over 40 *R* genes have been isolated from different rice germplasm, most of which encode nucleotide-binding domain leucine-rich repeat (NLR) proteins, except *Pid2*, *pi21* and *Ptr* (Chen et al. 2006; Fukuoka et al. 2009; Zhao et al. 2018; Fan et al. 2024; Jiang et al., 2024). Interestingly, 12 *Pi* genes, including 6 cloned ones *Pi9*, *Pi2*, *Piz-t*, *Pi50*, *Pigm* and *Pizh*, are located at the *Pi2/9/Pigm* locus (Qu et al. 2006; Zhou et al. 2006; Wang et al. 2012; Su et al. 2015; Deng et al. 2017; Wei et al. 2019; Xie et al. 2019). These *Pi* genes have been reported to confer broad-spectrum resistance to diverse rice blast isolates. *Pigm* was identified from a Chinese *indica* variety Gumei 4 (GM4), which has been used as a blast resistance donor for more than 50 years, and it is considered the most broad-spectrum *Pi* gene identified so far (Deng et al. 2017). There is a cluster of NLR genes at *Pigm* locus, in which *PigmR* is responsible for the resistance and *PigmS* suppresses resistance by attenuating the *PigmR* homodimerization. Moreover, *PigmR* and *PigmS* also have an opposite role on grain yield. *PigmR* decreases the grain weight leading to yield penalty, but *PigmS* increases the seed setting leading to increased yield. The expression of *PigmS* is epigenetically regulated, which balances the blast resistance and grain yield with *PigmR*. Further investigation indicated that *PigmR* directly interacts with the new RRM-domain transcription factor family PIBP1 to promote their nuclear accumulation to activate defense genes (Zhai et al. 2019). *PigmR* and allele *NLRs* also safeguard ethylene biosynthesis to synchronize pattern- and effector-triggered immunity (PTI and ETI) to ensure broad-spectrum blast resistance (Zhai et al. 2022).

Hybrid rice cultivation, adopted by over 40 countries, has made a significant contribution to global food security by substantially increasing rice production

(Yuan 2014). Three-line and two-line hybrid rice system were developed in 1974 and 1995, respectively. The two-line hybrid rice system, including photoperiod-sensitive genic male-sterile (PGMS) and thermo-sensitive genic male-sterile (TGMS) based system, has comparative advantages over the three-line hybrid rice system, such as easy male sterile line multiplication, no restriction with regard to the restorer line, easy use of inter-subspecific heterosis and lower cost (Ma and Yuan 2015). Longke638S (LK638S), which was developed in 2014, is an elite TGMS line known for its broad ecological adaptability, and desirable traits such as good plant architecture, high outcrossing rate, strong combining ability and superior grain quality. So far, a total of ninety-four LK638S-derived hybrid rice varieties have been national-approved and certificated. According to the National Agricultural Technology Extension Center statistics, the promotion area of eighteen main LK638S-derived hybrid varieties has got to 913,000 hectares and 801,000 hectares in 2022 and 2023, respectively. Of the LK638S-derived hybrid varieties, Longliangyou-Huazhan (LLYHZ) got through all of the four state regional trials (the middle and lower reaches of Yangtze River, upper reaches of Yangtze River, South China, and Wuling Mountainous area) with an average increased yield 7.3%, and certificated by the national new variety examination and approval committee (NNVEAC). Since its initial certification in 2015, LLYHZ has emerged as one of the top three widely cultivated hybrid rice varieties in China, with annual promotion areas of more than 510,518 hectares during 2018–2023. However, LK638S exhibits only moderate blast resistance, which hinders its extensive application in hybrid varieties development with high climate-resilient and disease-proof. Therefore, enhancing the blast resistance of LK638S is crucial for its further exploiting in hybrid rice production in China.

In this study, we integrated the broad-spectrum blast resistance locus *Pigm* into the LK638S through backcross and molecular marker-assisted selection (MAS). Our objective is to further enhance the blast resistance of LK638S and its derived hybrid varieties, thereby ensuring reliable rice production. The improved LK638S, named as Longzhen36S (LZ36S), and its derived hybrids exhibited enhanced blast resistance in both in-door and open-nursery. A total of sixteen LZ36S-derived hybrids varieties with enhanced blast resistance ability have been national or provincial approved and certificated during 2020–2023. LZ36S and its derived hybrids varieties exhibited great potential in hybrid rice production.

Materials and methods

Plant materials

LK638S, an elite and widely used *indica*-type TGMS line of China, was used as the recurrent parent. GM4, an *indica* rice variety containing *Pigm*, was used as the donor. Thirteen restorer lines and two varieties as controls were used for phenotype analysis of derived hybrids (Supplementary Table S1). In addition, 28 varieties including GM4 were used for testing the specificity of the newly developed marker.

Allele-specific markers and genotyping

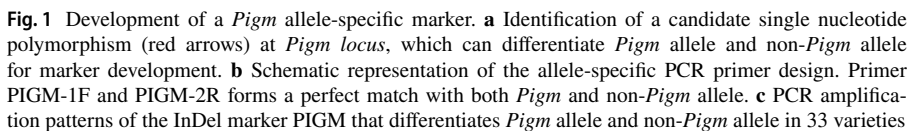
The genomic sequences of *Pi2/9/Pigm* locus in different cultivars were retrieved from GenBank database of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/) and Gramene (<http://www.gramene.org/>) using the accession numbers presented in previous papers (Zhou et al. 2007; Deng et al. 2017). DNA sequences were aligned using Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). According to the multiple sequence alignment, a candidate single nucleotide polymorphism (SNP), which can distinguish between *Pigm* and non-*Pigm* alleles, was identified. Based on this unique polymorphism, a *Pigm*-specific marker PIGM was developed for tracking the *Pigm* gene. In order to differentiate functional and nonfunctional *Hd1* allele, a marker HD1 targeting the InDel polymorphism between GM4 and LK638S was developed. All the primers used in this study were listed in Additional file 2: Table S2. Leaf tissues were collected and DNA was extracted from ground tissues according to the protocol described by Murray and Thomson with minor modification (Murray and Thompson 1980). The total volume of the polymerase chain reaction (PCR) reaction was 10 μ L, containing 1 μ L of 10 $\times$ PCR buffer, 0.1 mM dNTPs, 0.1 μ M of primer pairs, 0.1 U Taq DNA polymerase, 25 ng template DNA. Thermal cycles were programmed as follows: 94 $^{\circ}$ C, 3 min; 35 cycles of 95 $^{\circ}$ C for 30 s; 55 $^{\circ}$ C for 40 s; and 72 $^{\circ}$ C for 40 s, concluding with 72 $^{\circ}$ C for 5 min. The PCR products were electrophoresed in 4% agarose gel (Amresco, USA) stained with ethidium bromide and visualized using GelDocXR (Bio-Rad, USA).

Marker-assisted breeding strategy

The procedure of introgression of *Pigm* into LK638S is illustrated in Fig. 1. The recurrent parent LK638S was crossed as female parent with the donor, GM4. During the backcrossing process, selected individuals heterozygous at *Pigm* locus were backcrossed to LK638S. The allele-specific marker PIGM and HD1 were used to select plants carrying *Pigm* and *Hd1* locus in backcross populations, respectively. BC₃F₁ plants were selected using both PIGM and HD1 markers for the plants with *Pigm* and nonfunctional *Hd1*. The selected plants were selfed and backcrossed again. After continuous pedigree selection combined with marker-assisted selection, a newly-developed TGMS line carrying *Pigm* and nonfunctional *Hd1* was obtained.

Evaluation of rice blast resistance

Field evaluation of leaf and neck blast resistance was performed from April to November in the blast nursery of Daweishan, Hunan Province, China. A randomized complete block design was used in which 30 seedlings of each tested rice lines were transplanted in single-row plots with three replications. The highly susceptible varieties Xiangazao7 were planted on the plot borders. Disease



Twenty-eight *M. oryzae* isolates, collected from 7 provinces of China, French Guyana and South Korea, were used for inoculation experiments in greenhouse. Two-week-old seedlings (3–4 leaves) for each line were sprayed with 20 ml of the spore suspension at a concentration of 5×10^5 spores mL⁻¹, and then were moved into a chamber with 95% humidity under dark conditions at 27 °C for 24 h. After 24 h, the inoculated plants were maintained in the same room and the conditions were changed to provide 12 h light/12 h dark and 85% humidity for 6 days for disease development. The disease reactions were scored at 7 days post inoculation

with a 0 to 5 scale rating system. Scores 0–2 were considered resistant (R) and 3–5 as susceptible (S).

Fertility-sterility alteration test

Seeds were sowed in the paddy field at the end of May of 2016. Seedlings were then transferred to teen plastic buckets with 5 plants in each bucket during their five-leaf stage. During pollen formation stage, two seedling buckets were placed successively in 21 °C, 22 °C, 23 °C, 24 °C, and 25 °C water for 6 days, respectively. Subsequently, the pollen sterility of each panicle was recorded according to the classification of pollen morphology upon IK-I staining of pollen grains (Xu et al. 2002).

Evaluation of agronomic traits and grain quality

Agronomic traits of LZ36S, LK638S and their derived hybrids were evaluated in the paddy field, during the summer season of 2019 at the breeding station of Yahua Seeds Science Academy, Ningxiang (N28°16', E112°41'), Hunan Province, China. Each plot was comprised of eight rows with 10 plants per row at planting density of 17 cm between plants and 20 cm between rows. Five individuals in the middle of each plot were taken for measurements of agronomic traits, including days to heading (DTH), days to maturity (DTM), plant height (PH), panicle number (PN), panicle length (PL), number of grains per panicle (NGP), spikelet fertility (SF), thousand-grains weight (TGW), grain length (GL), grain width (GW) and grain yield (GY). Grain yield per plot was evaluated by harvesting the grains from 20 plants in the central row of each plot. One hundred fully filled dehulled grains were randomly selected to determine grain length, grain width, length to width ratio (LWR), head rice rate (HRR) and chalkiness rate (CR) using the Wanshen SC-E rice appearance quality detection analyzer (Hangzhou, China). The amylose content (AC), gel consistency (GC) and alkali spreading value (ASV) were also measured according to national or agricultural industry standard of the People's Republic of China (NY/T 2639, NY/T 83–2017, GB/T 22294).

Genetic background analysis based on whole-genome SNP array

Genomic DNA was prepared using the CTAB method as described by Murray and Thomson (Murray and Thompson 1980). A 1.2 K multiplex-PCR panel, which was based on GBTS platform from MolBreeding Biotechnol (<http://www.molbreeding.com>), was employed for background genotyping. A total of 48 SSR makers were also used in background genotyping, following the protocol of the identification of rice varieties by SSR marker method (NY/T 1433–2014).

Results

Allele-specific marker development of *Pigm*.

By conducting multiple sequence alignment of the *Pi2/9/Pigm* locus in various cultivars including GM4(*Pigm*), LK638S, 75–1–127(*Pi9*), C101A51(*Pi2*), Nipponbare and 93–11, we discovered a single nucleotide polymorphism (SNP) located at the 5' side of the locus, which has the potential to distinguish between *Pigm* and non-*Pigm* allele. Confronting two-pair primers targeted the region containing the identified SNP were designed to develop a marker PIGM (Supplementary Table S2). To assess the specificity of the PIGM marker, we analyzed 33 rice varieties. PCR results confirmed that only GM4 exhibited amplification of a 621 bp fragment, while the remaining 27 varieties lacking the *Pigm* allele displayed a 448 bp fragment (Fig. 1). Therefore, this functional allele-specific PCR-gel-based marker can be used for *Pigm* allele identification in rice breeding pipeline.

Introgression of *Pigm* by MAS

Pigm was transferred into an elite TGMS line LK638S by advanced-backcrossing combined with MAS. As shown in the schematic flow (Fig. 2), the donor GM4 harboring *Pigm* was used for backcrossing with the recurrent female parent LK638S. In the backcross generation, the F_1 plants showing male-sterile and harboring heterozygous *Pigm* gene were ratooned and transferred in water incubation with controlled temperature to produce pollens for the backcrossing process. Male-sterile plants

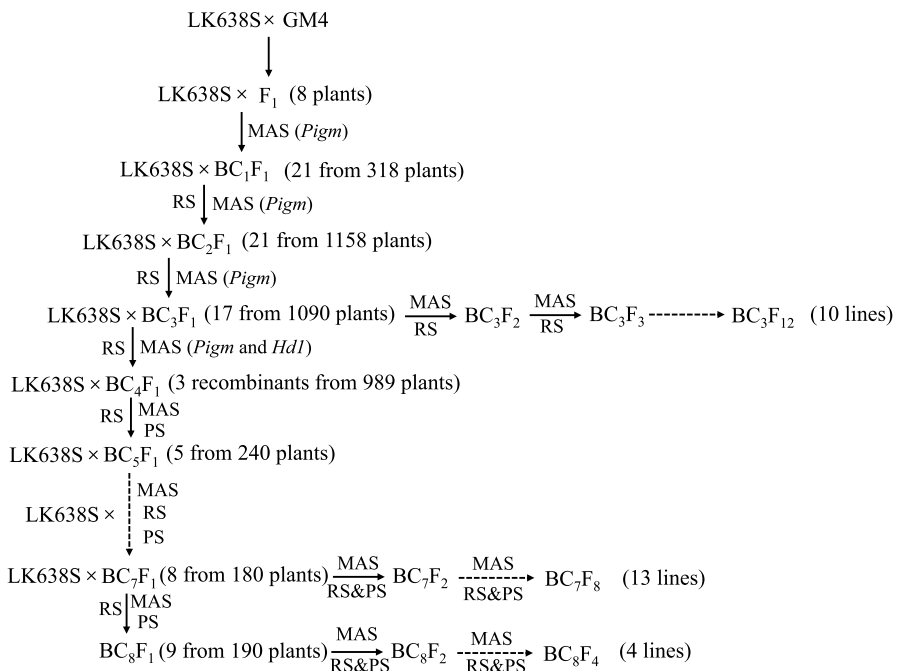


Fig. 2 Detailed scheme for introgression of *Pigm* into LK638S through marker-assisted selection (MAS), resistance selection (RS) and phenotypic selection (PS)

with homozygous *Pigm* were selected in BC₃F₂, BC₈F₂ and BC₉F₂ populations and ratooned in water incubation to obtain next generation seeds by selfing. This procedure was repeated to obtain advanced generations including BC₃F₁₂, BC₈F₈ and BC₉F₄. Blast resistance was evaluated in the Daweishan blast nursery (Liuyang county, Huan province, China), while phenotyping, including evaluation of fertility and agronomic performance, was conducted at each generation to select plants with desirable phenotypes. Finally, a total of 13 elite male sterile lines with homozygous *Pigm* were selected from the BC₈F₈ population, along with 4 lines from the BC₉F₄ population in 2018. In addition, 10 lines from BC₃F₁₂ were also included for further experimentation.

Genetic linkage-drag between blast resistance and photosensitivity

During the introgression of blast resistance gene *Pigm* by MAS, we observed that a delayed heading phenotype in the sterile plants carrying *Pigm*. Furthermore, we noticed that the majority of hybrids derived from the improved lines as female parents exhibited photoperiod sensitive, resulting in failure to head during rice-cropping season at Changsha (Hunan province, China). To figure out this particular phenomenon, we conducted genome sequencing of the region surrounding *Pigm*, and discovered that the CCT domain-containing protein gene *Hdl* is situated approximately 181.5 kb away from *Pigm*. *Hdl* is known to regulate the heading date in a photoperiod sensitivity manner (Yano et al. 2000). By comparing the *Hdl* sequences of LK638S and GM4, we observed that LK638S carried a loss-of-function allele of *Hdl*, while GM4 possessed the wild type (functional) *Hdl* allele. This indicates that the genetic linkage-drag between blast resistance and photoperiod sensitivity may be a result of the genetic linkage between *Pigm* and *Hdl*. To develop a desired photoperiod insensitive variety, it is indeed possible to eliminate this genetic linkage.

We designed functional molecular markers of *Hdl* based on a 4-bp InDel variation in CCT domain for MAS selection (Supplementary Fig. S1; Supplementary Table S2). In BC₄F₁ population, ~5000 plants were genotyped with the molecular markers of *Pigm* and *Hdl*, and nine recombinants with heterozygous *Pigm* and homozygous *Hdl*^{LK638S} were identified. Top three recombinants exhibiting phenotypes more similar to LK638S were selected for further backcrossing with LK638S. The lines with *Hdl*^{638S}-*Pigm* exhibited a significant reduction in days to heading (DTH) compared to those harbouring *Hdl*^{GM4}-*Pigm* (Table 1).

Evaluation of blast resistance in natural nursery and inoculation

The seedling blast resistance of the newly developed TGMS lines and two parents were evaluated in natural nursery and greenhouse conditions, respectively. The newly improved lines and donor GM4 showed stable and high-level seedling blast resistance without any typical lesions in the blast nursery trails for three consecutive years (2016–2018). In contrast, the parental line LK638S displayed susceptible to rice blast (Table 1). In the greenhouse, we inoculated the improved lines and parents with 28 isolates collected from different regions of China, French,

Table 1 Blast resistance and heading date of selected improved lines and parents

Cultivar/breeding lines	Generation	Haplotype	DTH	BR
LK638S	Recurrent parent	<i>Hd1</i> ^{638S}	94	S
GM4	Donor parent	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	87	R
X5-4-1021	BC ₂ F ₁₃	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	103	R
X5-4-768	BC ₂ F ₁₃	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	102	R
X7-7-1024	BC ₂ F ₁₃	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	101	R
X7-7-1031	BC ₂ F ₁₃	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	102	R
D23-924	BC ₂ F ₁₃	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	103	R
D23-933	BC ₂ F ₁₃	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	102	R
D5-123	BC ₃ F ₁₂	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	102	R
D5-141	BC ₃ F ₁₂	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	101	R
D5-205	BC ₃ F ₁₂	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	102	R
D5-269	BC ₃ F ₁₂	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	101	R
D5-333	BC ₃ F ₁₂	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	101	R
D5-397	BC ₃ F ₁₂	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	103	R
D5-425	BC ₃ F ₁₂	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	102	R
D5-461	BC ₃ F ₁₂	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	101	R
D5-478	BC ₃ F ₁₂	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	101	R
D5-487	BC ₃ F ₁₂	<i>Hd1</i> ^{GM4} - <i>Pigm</i>	102	R
LP3-6-904-2	BC ₈ F ₈	<i>Hd1</i> ^{638S} - <i>Pigm</i>	91	R
LP3-3-829-1	BC ₈ F ₈	<i>Hd1</i> ^{638S} - <i>Pigm</i>	84	R
LP3-6-900	BC ₈ F ₈	<i>Hd1</i> ^{638S} - <i>Pigm</i>	92	R
LP3-6-901-1	BC ₈ F ₈	<i>Hd1</i> ^{638S} - <i>Pigm</i>	93	R
LP3-6-902-2	BC ₈ F ₈	<i>Hd1</i> ^{638S} - <i>Pigm</i>	91	R
LP3-5-820	BC ₈ F ₈	<i>Hd1</i> ^{638S} - <i>Pigm</i>	95	R
LP3-6-901-2	BC ₈ F ₈	<i>Hd1</i> ^{638S} - <i>Pigm</i>	90	R
LP3-6-896-1	BC ₈ F ₈	<i>Hd1</i> ^{638S} - <i>Pigm</i>	92	R
LP3-3-832-1	BC ₈ F ₈	<i>Hd1</i> ^{638S} - <i>Pigm</i>	85	R
LP3-6-805-2	BC ₈ F ₈	<i>Hd1</i> ^{638S} - <i>Pigm</i>	92	R
LP3-6-902-2	BC ₈ F ₈	<i>Hd1</i> ^{638S} - <i>Pigm</i>	92	R
LP3-6-902-4	BC ₈ F ₈	<i>Hd1</i> ^{638S} - <i>Pigm</i>	92	R
LP3-6-899-1	BC ₈ F ₈	<i>Hd1</i> ^{638S} - <i>Pigm</i>	91	R
A145-5-1	BC ₉ F ₄	<i>Hd1</i> ^{638S} - <i>Pigm</i>	91	R
A145-5-4	BC ₉ F ₄	<i>Hd1</i> ^{638S} - <i>Pigm</i>	96	R
A145-5-6	BC ₉ F ₄	<i>Hd1</i> ^{638S} - <i>Pigm</i>	93	R
A145-5-8	BC ₉ F ₄	<i>Hd1</i> ^{638S} - <i>Pigm</i>	93	R

DTH Days to heading; *BR* Leaf blast resistance over multiple years at the Daweishan blast nursery

Guyana and South Korea. CO39 (susceptible control) and LK638S showed resistance frequency of 0% and 57.1%, respectively, indicating the presence of other blast resistance gene(s) in LK638S. The donor GM4 was immune to all of the 28 isolates. The 12 newly developed TGMS lines showed significantly enhanced

blast resistance, with frequencies ranging from 92.9% to 96.4% (Supplementary Table S3).

Assessment of fertility-sterility alternation characterization and agronomic traits

The fertility-sterility alternation of the 12 improved TGMS lines and the recurrent parent LK638S was assessed in a water-filled concrete tank with controlled water temperature. The experiment was conducted in August 2019, with daily mean water temperatures (DWT) set at 21 °C, 22 °C, 23 °C, 24 °C, and 25 °C. The results showed that both the improved TGMS lines and LK638S exhibited complete male sterility, with 100% pollen sterility observed when the daily mean water temperature (DWT) reached 23 °C. Partial fertility was observed under 22 °C (Table 2). The pollen fertility-sterility alternation pattern of the improved TGMS lines was similar to that of the recurrent parent LK638S, with the critical temperature point for fertility-sterility alternation occurring between 22 °C and 23 °C of DWT.

Twelve newly developed TGMS lines were also evaluated for their agronomic traits at Changsha (China) in the summer of 2019. The results showed no significant differences for all tested traits between newly developed TGMS lines and LK638S (Supplementary Table S4; Fig. S2).

Genomic evaluation of improved TGMS lines

A 1.1 K multiplex-PCR panel, based on the GBTS platform, was used for genomic background genotyping of the newly improved TGMS lines. Of the 1125 SNP markers covering 12 chromosomes, 264 SNPs showing polymorphism between the donor GM4 and recipient LK638S were used for genomic recovery analysis of improved

Table 2 Fertility-sterility alternation behavior of improved lines and the recurrent parent under five different temperature regimes

Cultivar/lines	Pollen sterility (%)				
	21°C/6d	22°C/6d	23°C/6d	24°C/6d	25°C/6d
LK638S	58.03	90.94	100.00	100.00	100.00
LP3-3-829-1	41.80	85.00	100.00	100.00	100.00
LP3-3-832-1	44.20	84.40	100.00	100.00	100.00
LP3-6-805-3	54.00	89.70	100.00	100.00	100.00
LP3-6-896-1	52.30	89.50	100.00	100.00	100.00
LP3-6-899-1	52.80	87.90	100.00	100.00	100.00
LP3-6-901-2	52.48	88.19	100.00	100.00	100.00
LP3-6-902-4	53.77	89.51	100.00	100.00	100.00
LP3-6-904-2	54.30	90.09	100.00	100.00	100.00
A145-5-1	54.80	89.23	100.00	100.00	100.00
A145-5-4	54.70	89.11	100.00	100.00	100.00
A145-5-6	55.17	89.07	100.00	100.00	100.00
A145-5-8	55.44	89.31	100.00	100.00	100.00

TGMS lines. The newly improved TGMS lines showed a high genome recovery rate of the recurrent parent at 89.58%–95.83% with the introgression harbouring *Pigm* of approximately 1.92–8.52 Mb (Supplementary Table S5). Additionally, the genetic background of the newly improved TGMS lines were also evaluated with 48 SSR makers, which are commonly used for the rice varieties identification according to the Agricultural industry standard of the People's Republic of China (NY/T 1433–2014). The results revealed only 0–3 polymorphisms between the improved lines and the parent LK638S (Supplementary Table S5).

For further verification, whole-genome resequencing data were generated for GM4, LK638S, LP3-6-899-1 and LP3-6-902-4 with an average minimum coverage of 26.0X, resulting in a total of 3,272,591 SNPs for background genotyping of LP3-6-899-1 and LP3-6-902-4. A graphical genotype map was constructed and assumed that the introgressed chromosome segment containing the *Pigm* locus of LP3-6-899-1 and LP3-6-902-4 were approximately 7.2 and 8.1 Mb, respectively, with a genome recovery rate of 92.07% and 91.84% for the recurrent parent (Supplementary Fig. S3; Supplementary Table S5).

Evaluation of blast resistance and agronomic traits of LZ36S derived hybrids

Based on the comprehensive evaluation of blast resistance, fertility-sterility alternation characterization, agronomic traits, combining ability and genetic background profiling of the newly improved TGMS lines, the improved line F3-6-902-4 was selected for further breeding application and named LZ36S (Fig. 3a). LZ36S and LK638S were used as female parents to produce hybrid F₁ seeds with 13 male parents. All male parents, except for R1988, harbour *Pi2* allele. All 13 hybrids of LK638S have been national certificated as new hybrid rice varieties and promoted extensively in Southern-China. Such as, Longliangyou-Huazhan (LK638S/Huazhan), a hybrid rice variety with wide-adaptability, has been national approved in all the major rice cropping region of Southern-China and can be cropped as middle-season rice in the whole Yangtze River region and the Wuling mountain area, as well as late-season rice in south China. Field blast resistance of the 26 hybrids was assessed in the Daweishan blast nursery with high blast disease pressure in 2019. The LZ36S-derived hybrids all displayed improved seedling and panicle blast resistance compared to the corresponding control hybrid of LK638S with same male parents (Table 3; Fig. 3b). It is also verified that, due to the difference in resistance spectra between *Pigm* and *Pi2* (Deng et al. 2017; Liu et al. 2002), and the ability of hybrid rice to aggregate two alleles from the same gene cluster, the broad-spectrum resistance of hybrid rice be further enhanced by aggregating and utilizing *Pigm* and *Pi2* (Table 3; Fig. 3b).

Yield performance of the hybrids of LZ36S and LK638S was field-trialed at the Guanshan experimental station under blast-control condition and the Daweishan blast nursery under open-field blast infection condition in 2019. The results showed that the average yield of the LZ36S-derived hybrids was 10.95 t/ha under blast-control condition, slightly lower than the yield (11.00 t/ha) of LK638S-derived hybrids. However, under open-field blast infection condition, the yield performance of the

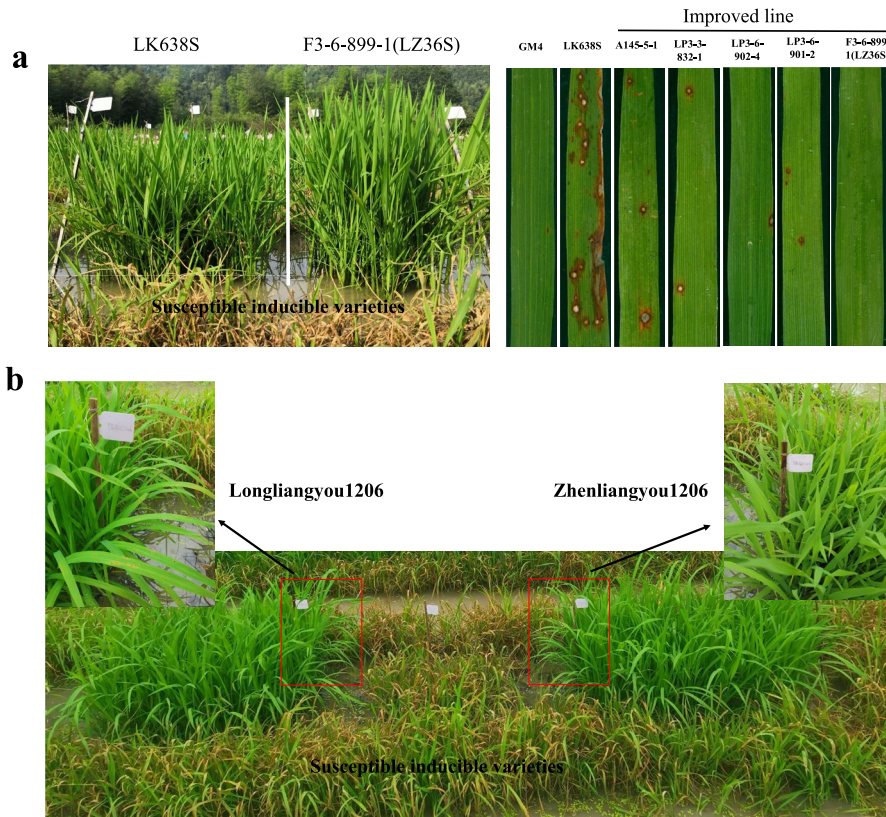


Fig. 3 Blast resistance reaction of selected improved lines and parents (a), Longliangyou1206 and Zhenliangyou1206 (b) in open-field blast nursery

LZ36S-derived hybrids was significantly improved, the average yield increased by 15.53% compared with that of LK638S-derived hybrids. We analysed six agronomic traits (DTM, PH, PNH, NGP, SF and TGW) of the hybrids trailed in the Guanshan experimental station. Compared with LK638S-derived hybrids, LZ36S-derived hybrids exhibited decrease in DTM, PNH, and NGH by an average of -0.13% , -3.46% , and -3.53% respectively. While, PH, SF and TGW increased 0.88% , 0.06% and 0.64% , respectively (Supplementary Table S6). These results indicated that the hybrids with the more major blast resistance gene allele (*Pigm*) possessed higher stable production capacity and no or minimal penalty on yield, although the hybrids also harbored another major broad-spectrum blast resistance allele of *Pi2*. This also highlights the effectiveness of utilizing double alleles of one favorable R gene present in both parental lines through hybrid rice system. From 2020 to 2023, a total of nineteen LZ36S-derived hybrid varieties, all exhibiting MR or R blast, and even HR resistance ability, along with favorable yield and grain quality performance, have been national or/and provincial approved and certificated (Supplementary Tables S7-S8). In 2024, the promoting area of LZ36S-derived hybrid

Table 3 Variety information and blast resistance performance of the hybrids derived from LK638S and LZ36S with same male parent

Hybrid variety	Combination	Blast resistance gene at <i>Pigm/Pi2</i> in female/male parent	Seedling blast resistance score	Panicle blast score	State Approval Variety No	Approved promotion area	Annotation
LongliangyouHuazhan	LK638S/Huazhan	-/ <i>Pi2</i>	4	5	No.2015026 No.2016045 No.20170008	MRMLY MRWL MRMLY; LRSC	Super rice recognized by Ministry of Agriculture and Rural Affairs of China in 2017; Green super rice recognized by green super-rice committee (China) in 2019; The top five largest promoted hybrid variety in 2018–2022 with annual promotion area 239.6 thousand hectares, 158.0 thousand hectares in 2023
ZhenliangyouHuazhan	LZ36S/Huazhan	<i>Pigm/Pi2</i>	2	3	-		
Longliangyoul 686	LK638S/R1686	-/ <i>Pi2</i>	5	5	No.20186058	MRMLY	
Zhenliangyoul 686	LZ36S/R1686	<i>Pigm/Pi2</i>	2	3	-		
Longliangyoul 273	LK638S/R1273	-/ <i>Pi2</i>	5	5	No.20186065	MRMLY	
Zhenliangyoul 273	LZ36S/R1273	<i>Pigm/Pi2</i>	3	3	-		

Table 3 (continued)

Hybrid variety	Combination	Blast resistance gene at <i>Pigm/Pi2</i> in female/male parent	Seedling blast resistance score	Panicle blast score	State Approval Variety No	Approved promotion area	Annotation
Longliangyou1308	LK638S/R1308	-/ <i>Pi2</i>	4	5	No.20176065	MRMLY	Super rice recognized by Ministry of Agriculture and Rural Affairs of China in 2019; The 35st, 63th and 110th largest promoted hybrid variety in 2021, 2022 and 2023, respectively, with annual 27.0 thousand hectares
Zhenliangyou1308	LZ36S/R1308	<i>Pigm/Pi2</i>	3	3	-		
Longliangyou7810	LK638S/R7810	-/ <i>Pi2</i>	4	5	No.20186009	MRUY; MRMLY	
Zhenliangyou7810	LZ36S/R7810	<i>Pigm/Pi2</i>	3	1	-		
Longliangyou1234	LK638S/R1234	-/ <i>Pi2</i>	5	5	No.20186011	MRMLY	
Zhenliangyou1234	LZ36S/R1234	<i>Pigm/Pi2</i>	3	3	-		
Longliangyou1206	LK638S/R1206	-/ <i>Pi2</i>	4	5	No.2016606	MRUY	
					No.20176009	MRMLY	
Zhenliangyou1206	LZ36S/R1206	<i>Pigm/Pi2</i>	2	1	-		

Table 3 (continued)

Hybrid variety	Combination	Blast resistance gene at <i>Pigm/Pi2</i> in female/male parent	Seedling blast resistance score	Panicle blast score	State Approval Variety No	Approved promotion area	Annotation
Longliangyoul 212	LK638S/R1212	-/ <i>Pi2</i>	5	5	No.20170022 No.20196013	MRMLY; MRWL MRUY; LRSC	Super rice recognized by Ministry of Agriculture and Rural Affairs of China in 2019; The 42st, 40th and 97th largest promoted hybrid variety in 2021, 2022 and 2023, respectively, with annual 22.0 thousand hectares
Zhenliangyoul 212	LZ36S/R1212	<i>Pigm/Pi2</i>	3	3	-		Super rice recognized by Ministry of Agriculture and Rural Affairs of China in 2019; The 17st, 24st and 36st largest promoted hybrid variety in 2021, 2022 and 2023, respectively, with annual 74.9 thousand hectares
Longliangyoul 377	LK638S/R1377	-/ <i>Pi2</i>	4	5	No.20176007	MRMLY; LRSC	
Zhenliangyoul 377	LZ36S/R1377	<i>Pigm/Pi2</i>	3	1	-		

Table 3 (continued)

Hybrid variety	Combination	Blast resistance gene at <i>Pigm/Pi2</i> in female/male parent	Seedling blast resistance score	Panicle blast score	State Approval Variety No	Approved promotion area	Annotation
Longliangyou1988	LK638S/R1988	-/-	7	7	No.2016609 No.20176010	MRMLY MRUY; LRSC	Super rice recognized by Ministry of Agriculture and Rural Affairs of China in 2018; The 33st, 45st and 57st largest promoted hybrid variety in 2021, 2022 and 2023, respectively, with annual 33.6 thousand hectares
Zhenliangyou1988	LZ36S/R1988	<i>Pigm</i> /-	4	5	-		The top ten largest promoted hybrid variety in 2018–2023 with annual promotion area 229.6 thousand hectares
Longliangyou534	LK638S/R534	-/ <i>Pi2</i>	4	5	No.2016603 No.20170001	LRSC MRUY; MRMLY	
Zhenliangyou534	LZ36S/R534	<i>Pigm</i> / <i>Pi2</i>	2	1	-		Super rice recognized by Ministry of Agriculture and Rural Affairs of China in 2022. The 55st largest promoted hybrid variety with 40.7 thousand hectares in 2023
Longliangyou5438	LK638S/R5438	-/ <i>Pi2</i>	5	5	No.20196050 No.20210012	MRML ERSC	

Table 3 (continued)

Hybrid variety	Combination	Blast resistance gene at <i>Pigm/Pi2</i> in female/male parent	Seedling blast resistance score	Panicle blast score	State Approval Variety No	Approved promotion area	Annotation
Zhenliangyou5438	LZ36S/R5438	<i>Pigm/Pi2</i>	3	3	No.20206138 No.20220010	MRMLY MRWL	The 350st largest promoted hybrid variety with 8.7 thousand hectares in 2023
Longliangyou8612	LK638S/R8612	-/ <i>Pi2</i>	4	5	No.20190010	MRMLY	Super rice recognized by Ministry of Agriculture and Rural Affairs of China in 2023; The 32st, 14th and 7st largest promoted hybrid variety in 2021, 2022 and 2023, respectively, with annual 92.2 thousand hectares
Zhenliangyou8612	LZ36S/R8612	<i>Pigm/Pi2</i>	2	3	No.20206139	MRMLY	The 17st largest promoted hybrid variety with 108.0 thousand hectares in 2023
GM4			3	3			
CA101A51			3	3			

MRMLY Middle-season rice in the middle and lower reaches of the Yangtze River; *MRUY* Middle-season rice in the upper reaches of the Yangtze River; *LRSC* Late-season rice in South China; *ERSC* Early-season rice in South China; *MRWL* Middle-season rice in Wuling mountainous area. The variety promoting data was from National Agricultural Technology Extension Center. Evaluation of blast resistance was conducted at the Daweishan blast nursery in 2019

varieties is expected to 320 thousand hectares, and Zhenlaingyou8612 (LZ36S/R8612) exceeded 200 thousand hectares.

Discussion

The development of rice varieties with durable and broad-spectrum resistance is of utmost crucial in combating diseases, ensuring food security and sustainable rice production. Between 2001 to 2020, a total of 1849 *indica* hybrid varieties were national approved and certificated. However, only 260 varieties of these varieties (14.1%) showed resistant or moderate-resistant to blast in new variety trial. This highlights the urgent demand to enhance blast resistance in hybrid rice varieties.

LK638S is a highly valuable TGMS line developed by Yuan Longping High-Tech, demonstrating significant commercial potential. Since its release in 2014, a total of ninety LK638S-derived new hybrid varieties have been approved and certificated. However, along with its commercialization, LK638S, despite initially displaying moderate resistance to blast, has shown declining blast resistance in recent years. This greatly threatens its utilization in hybrid rice production. Improving LK638S with enhanced blast resistance is thus a priority breeding program. Practically, it is often difficult to transfer blast resistance genes from donor germplasm into an elite modern variety by conventional breeding methods due to the unexpected linkage-drags. MAS breeding offers a highly efficient measure that enables precise transfer and pyramiding of target genes. In this study, we effectively introduced a broad-spectrum and durable blast resistance gene *Pigm* into an elite TGMS line LK638S using MAS. This was achieved through the precise selection with functional molecular markers and genomic background selection extensively with SNP chip and SSR markers. This breeding procedure ensures a high level of recurrent parent genome recovery while incorporating the desired traits.

During the improvement of LK638S, an unexpected linkage-drag between *Pigm* and heading date was observed. The improved lines with *Pigm* showed delayed heading and most derived hybrids exhibited photoperiod sensitivity, resulting in delayed or absent heading during rice-cropping season. Through comprehensive genetic and genomic analysis revealed that the functional allele of *Hd1* adjacent to *Pigm* in GM4 as the cause of this delayed heading drag, while LK638S carried a loss-of-function allele of *Hd1*. To overcome this unexpected linkage-drag, we employed MAS and a large segregating population to break down the tight linkage-drag of *Pigm* and *Hd1* within 200 kb. This resulted in the development of a new elite line LZ638S, which not only possesses high blast resistance but also exhibits a desirable heading date. By successfully removing the linkage-drag, LZ638S-derived hybrids offer enhanced blast resistance and maintain normal heading during the rice-cropping season in Changsha. LK638S likely carries at least four other blast resistance genes, such as *Pia*, *Piz*, *Pid2* and *Pid3* (unpublished data), which provide moderate blast resistance with a frequency of 57.1%. In contrast, the newly developed TGMS lines showed significantly improved blast resistance in open-field blast nursery and resistance frequency with 92.9% to 96.4% of 28 isolates in in-door inoculation. Four improved

TGMS lines, LP3-6-896-1, LP3-6-899-1, LP3-6-902-4 and LP36904-2, exhibited the most favourable comprehensive performance with fertility-sterility alternation temperature between 22 °C to 23 °C and similar or improved agronomic traits compared with the recurrent parent LK638S. LZ36S harbours *Hdl*^{638S}-*Pigm*^{GM4} of 8.1 Mb and with genome recovery rate of 91.84%. The hybrid varieties of LZ36S have the potential to serve as updated versions of blast resistance hybrid varieties, replacing current hybrid varieties of LK638S. This indicates that combining different favourable resistance alleles from both parents through molecular design is a practical approach to pyramid multiple desirable alleles and further enhance the blast resistance of a hybrid variety. Currently, a total of 16 new hybrid varieties of LZ36S with high blast resistance, favorable yield and grain quality performance have been approved and certificated. One such new variety, Zhenlaingyou8612 (LZ36S/R8612) approved in 2020, has reached an expected promotion exceeding 200,000 hectares by 2024. Another one, ZhenlaingyouYuzhan (LZ36S/Yuzhan), received approval in 2021, and its promotion area is expected to exceed 50,000 hectares in 2024. The promotion area for LZ36S hybrid varieties should further ensuring rice production in the future. This study further demonstrates that MAS breeding can effectively enhance the efficiency of gene aggregation in varieties improvement, particularly when genes are linked with unfavorable alleles. Moreover, it underscores the breeding value of *Pigm*.

Conclusions

To further improve blast resistance of the elite TGMS line LK638S and its derived hybrid rice varieties, LZ36S with *Pigm* introgression was developed by advanced backcrossing, marker-assisted and phenotypic selection. During the development of LZ36S, a genetic linkage-drag between blast resistance and photosensitivity was identified, which is caused by the *Hdl* gene. By incorporating the *Hdl*^{638S}-*Pigm* allele, LZ36S shows high and broad-spectrum resistance to rice blast while maintaining normal heading performance. Blast resistance of the LZ36S-derived new hybrids is thereby improved. Currently, sixteen LZ36S-derived new hybrid varieties, all with MR or R levels of blast resistance, have been certificated and demonstrated great potential for commercialization.

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files (Supplementary Tables S1, S2, S3, S4, S5, S6, S7 and S8; Figures S1 and S2).

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication All authors provided the consent for publication.

Competing interests The authors declare no competing interests.

References

- Chen X, Shang J, Chen D et al (2006) AB-lectin receptor kinase gene conferring rice blast resistance. *Plant J* 46:794–804. <https://doi.org/10.1111/j.1365-3113X.2006.02739.x>
- Deng Y, Zhai K, Xie Z et al (2017) Epigenetic regulation of antagonistic receptors confers rice blast resistance with yield balance. *Science* 355:962–965. <https://doi.org/10.1126/science.aai8898>
- Fan Y, Ma L, Pan X et al (2024) Genome-wide association study identifies rice panicle blast-resistant gene Pb4 encoding a wall-associated kinase. *Int J Mol Sci* 25:830. <https://doi.org/10.3390/ijms25020830>
- Fukuoka S, Saka N, Koga H et al (2009) Loss of function of a proline-containing protein confers durable disease resistance in rice. *Science* 325:998–1001. <https://doi.org/10.1126/science.1175550>
- Liu G, Lu G, Zeng L et al (2002) Two broad-spectrum blast resistance genes, *Pi9* (*t*) and *Pi2* (*t*), are physically linked on rice chromosome 6. *Mol Genet Genomics* 267:472–480. <https://doi.org/10.1007/s00438-002-0677-2>
- Ma GH, Yuan LP (2015) Hybrid rice achievements, development and prospect in China. *J Integr Agr* 14:197–205. [https://doi.org/10.1016/S2095-3119\(14\)60922-9](https://doi.org/10.1016/S2095-3119(14)60922-9)
- Murray MG, Thompson W (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res* 8:4321–4326. <https://doi.org/10.1093/nar/8.19.4321>
- Qu S, Liu G, Zhou B et al (2006) 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* 172:1901–1914. <https://doi.org/10.1534/genetics.105.044891>
- Skamnioti P, Gurr S (2009) Against the grain: safeguarding rice from rice blast disease. *Trends Biotechnol* 27:141–150. <https://doi.org/10.1016/j.tibtech.2008.12.002>
- Su J, Wang W, Han J, Chen S et al (2015) Functional divergence of duplicated genes results in a novel blast resistance gene *Pi50* at the *Pi2/9* locus. *Theor Appl Genet* 128:2213–2225. <https://doi.org/10.1007/s00122-015-2579-9>
- Takeda S, Matsuoka M (2008) Genetic approaches to crop improvement: responding to environmental and population changes. *Nat Rev Genet* 9:444–457. <https://doi.org/10.1038/nrg2342>
- Tanweer FA, Rafii MY, Sijam K et al (2015) Current advance methods for the identification of blast resistance genes in rice. *Comptes Rendus Biol* 338:321–334. <https://doi.org/10.1016/j.crv.2015.03.001>
- Wang Y, Wang D, Deng X et al (2012) Molecular mapping of the blast resistance genes *Pi2-1* and *Pi51(t)* in the durably resistant rice Tianjingyeshengdao. *Phytopathology* 102:779–786. <https://doi.org/10.1094/PHYTO-03-12-0042-R>
- Wang B, Ebbole D, Wang Z (2017) The arms race between *Magnaporthe oryzae* and rice: Diversity and interaction of *Avr* and *R* genes. *J Integr Agr* 16:2746–2760. [https://doi.org/10.1016/S2095-3119\(17\)61746-5](https://doi.org/10.1016/S2095-3119(17)61746-5)

- Wei X, Zeng Y, Zhang R et al (2019) Fine mapping and identification of the rice blast-resistance locus *Pi-kf2(t)* as a new member of the *Pi2/Pi9* multigene family. *Mol Breed* 39:108. <https://doi.org/10.1007/s11032-019-1017-0>
- Xie Z, Yan B, Shou J, Tang J et al (2019) A nucleotide-binding site-leucine-rich repeat receptor pair confers broad-spectrum disease resistance through physical association in rice. *Philos T R Soc B* 374:20180308. <https://doi.org/10.1098/rstb.2018.0308>
- Xu ML, Wu HX, Chen LB et al (2002) Studies on identifying the critical sterility-inducing temperature of PTGMS lines in rice with temperature controlled cold water bed (in Chinese). *Hybrid Rice* 17:51–53. <https://doi.org/10.3969/j.issn.1005-3956.2002.05.023>
- Yano M, Katayose Y, Ashikari M et al (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS*. *Plant Cell* 12:2473–2483. <https://doi.org/10.1105/tpc.12.12.2473>
- Yuan LP (2014) Development of hybrid rice to ensure food security. *Rice Sci* 21:1–2. [https://doi.org/10.1016/S1672-6308\(13\)60167-5](https://doi.org/10.1016/S1672-6308(13)60167-5)
- Zeigler R, Leong SA, Teng PS (1994) Rice blast disease. Oxford University Press, Wallingford
- Zhai K, Deng Y, Liang D et al (2019) Transcription Factors Interact with NLRs and Regulate Broad-Spectrum Blast Resistance in Rice. *Mol Cell* 74:996–1009. <https://doi.org/10.1016/j.molcel.2019.03.013>
- Zhai K, Liang DI, Li H et al (2022) NLRs guard metabolism to coordinate pattern-and effector-triggered immunity. *Nature* 601:245–251. <https://doi.org/10.1038/s41586-021-04219-2>
- Zhao H, Wang X, Jia Y et al (2018) The rice blast resistance gene *Ptr* encodes an atypical protein required for broad-spectrum disease resistance. *Nat Commun* 9:2039. <https://doi.org/10.1038/s41467-018-04369-4>
- Zhou B, Qu S, Liu G et al (2006) 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* 19:1216–1228. <https://doi.org/10.1094/MPMI-19-1216>
- Zhou B, Dolan M, Sakai H, Wang GL (2007) The genomic dynamics and evolutionary mechanism of the *Pi2/9* locus in rice. *Mol Plant-Microbe Interact* 20:63–71. <https://doi.org/10.1094/MPMI-20-0063>

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