

Molecular Breeding of a Novel PTGMS Line of Wdr for Broad-spectrum Resistance to Blast Using *Pi9*, *Pikh*, and *Pi5* Genes

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Abstract

Background: The two-line method based on the photoperiod and thermo-sensitive genic male sterile (PTGMS) lines is more cost-effective, simple, and efficient than the three-line system based on cytoplasmic male-sterility (CMS). Blast and drought are the most prevalent biotic and abiotic stress factors that hamper rice production, respectively. Molecular techniques demonstrate higher efficacy in the pyramiding of disease resistance genes, providing green performance under the background of water-saving and drought-resistance rice.

Results: This work employed molecular marker-assisted selection (MAS), conventional hybridization and high-intensity stress screening to integrate the broad-spectrum blast resistance genes *Pi9*, *Pikh*, and *Pi5* into Huhan 1S. Subsequently a novel water-saving and drought-resistance rice (WDR) PTGMS line Huhan 74S was developed. The drought resistance of the new PTGMS line Huhan 74S was comparable to that of Huhan 1S. The artificial inoculation of 14 blast strains revealed that the resistance frequency of Huhan 74S was 85.7%. Based on the conditions of natural field induction, Huhan 74S and its hybrid combination revealed satisfactory resistance to leaf and neck blast. The identification outcomes of photo-thermal characteristics showed that the critical point of Huhan 74S fertility conversion had an average daily temperature of 23°C, and the stable sterile period in Shanghai lasted 51 days. The rice quality of Huhan 74S was grade 3 based on standards issued by the ministry. Also, both the agronomic and rice quality performances adhered to the conditions of two-line hybrid rice production.

Conclusion: The newly bred PTGMS line Huhan 74S demonstrated a stable and lasting resistance to blast. Moreover, the hybrid combination showed a high yield potential and can be used in the breeding of high-yield, high-quality, disease-resistance two-line hybrid water-saving and drought-resistance rice (WDR), hence promoting sustainable rice production in China.

Introduction

Rice blast caused by *Magnaporthe oryzae* is a global rice disorder, and it severely affects the quality and yield of rice. The annual loss of rice production caused by blast is as high as hundreds of millions of kilograms across the globe (Skamnioti and Gurr, 2009), thereby significantly jeopardizing food security. Breeding and planting various disease-resistance rice are the most economical and efficient measures to control blast. Because of numerous physiological species and rapid variation of the blast bacteria, the loss of resistance can readily occur. It is extremely difficult to select broad-spectrum disease-resistance varieties via conventional breeding methods. There is a greater risk of using single-gene to prevent and control diseases, likely causing the loss of single-gene resistance hence the occurrence of larger diseases. As such, pyramiding of multiple disease resistance genes is the most feasible and effective approach for solving the current hazard of blast (Orasen et al, 2020). So far, at least 100 major genes of blast resistance have been identified and mapped in different rice germplasm resources. Wherein 27 genes including *Pita*, *Pib*, *Pb1*, *Pizt*, *Pid2*, *Pii*, *Pikm*, *Pit*, *Pid3*, *Pid3-A4*, *Pish*, *Pik*, *Pikp*, *Pia*, *PiCO39*, *Pi1*, *Pi2*, *Pi5*, *Pi9*, *Pi21*, *Pi25*, *Pi33*, *Pi36*, *Pi37*, *P50*, *Pi54*, and *Pi65 (t)* have been cloned. Also, more than 350 quantitative trait loci associated with rice blast resistance have been identified (Khanna et al, 2015; Zheng et al, 2016; Luo et al, 2017), providing a possibility of using molecular markers to detect these genes. Molecular marker-assisted selection (MAS) technology screens target traits in advance and enhance breeding efficiency (Tanksley et al. 1989). Notably, the *Pi9* gene confers broad spectrum blast disease resistance in rice. It is a similar multiple allele at the *Piz* locus as *Pi2*, *Pi50*, *Pigm*, *Piz*, and *Piz-t*. The 43 rice blast strains detected from 13 countries showed significantly high resistance (Liu et al, 2002). *Pikh*, a vital gene at the *Pik* locus is located on rice chromosome 11, and has a strong resistance to species from Yunnan, Sichuan, Guizhou, Guangdong, and other regions of China (Yang et al, 2008; Zhai et al, 2014; Zhang et al, 2017). *Pi5* gene, located on chromosome 9 and showed complete resistance to five rice blast species including PO6-6 under artificial inoculation conditions in the greenhouse, with a broad resistance spectrum to the blast strains from rice regions in Northeast China (Lee et al, 2009). These blast resistance genes are significantly valuable in rice breeding for disease resistance.

Photoperiod and thermo-sensitive genic male sterile line (PTGMS) regulate production in China and has significantly promoted the increase in rice yield (Yuan, 2017). Nonetheless, most PTGMS lines have poor resistance to blast (Dong et al, 2010). Related studies show that the disease resistance of hybrid rice is significantly and positively correlated with that of sterile lines (Jiang et al, 2012). In recent years, with the deterioration of the climate environment, drought has emerged as an important limiting factor for agricultural production. Therefore, cultivating water-saving and drought-resistance rice (WDR) is one of the critical strategies to addressing water shortage issues and increasing the yield of low- and medium-yield fields in China (Luo, 2010). The two-line sterile line bred in production lacks the characteristics of water-saving and drought-resistance. Breeding history and genomic studies indicate that lowland paddy rice and upland rice hybridization breeding with suitable selection in different environments is an effective approach to improving complex traits such as yield potential and drought resistance. Meanwhile, molecular technology has demonstrated higher efficiency in value-added breeding such as transfer and pyramiding of disease and insect resistance genes. This provides other green traits under the WDR background (Luo et al, 2019). Huhan 1S is a WDS PTGMS line of hybridization breeding using Y58S and Huhan 1B hybridization, with strong drought resistance and general blast resistance. We adopted MAS and conventional hybridization combined with high-intensity stress selection in the target to integrate the broad-spectrum blast resistance genes *Pi9*, *Pikh*, and *Pi5* into Huhan 1S. Subsequently, a novel PTGMS line Huhan 74S with blast resistance, strong drought resistance, excellent rice quality, and satisfactory combining ability was selected. This line passed technical appraisal test by the Shanghai Seed Management Station in August 2018, thereby providing an effective parental basis for further grouping of novel combinations of disease-resistance two-line hybrid WDR.

Results

Breeding process

In the winter of 2012, the Huhan 1S female parent and Huhan 1B intermediate male parent carrying the *Pi9* gene were used for hybridization. The plants identified as true hybrids by molecular markers in Shanghai in the summer of 2013 were used as female parents, while the japonica WDR Huhan 91 (*Pikh+Pi5*) was used as the male parent, and triple hybrids were obtained after hybridization. F₁ was planted in Hainan during the winter of 2013, and false hybrids were

removed via molecular marker identification, where more than 5,000 seeds were harvested. F₂ generation segregation population was planted in Shanghai during the summer of 2014. Based on the MAS of the blast-resistant genes *Pi9*, *Pikh*, and *Pi5*, we selected and segregated 27 sterile single plants with pure three genes, good comprehensive traits, and pollen-free microscopic examination. Then, the regenerated seeds were harvested. In the winter of 2014, the F₃ generation was planted in the dry land of Hainan, and 128 single plants with good comprehensive agronomic traits and strong drought resistance were selected. The F₄ generation was planted in Shanghai in the summer of 2015, then, combined with blast screening and agronomic trait inspection, 26 individual plants were selected, segregated and the regenerated seeds were harvested. Planting of the F₅ generation was continued in Hainan during the winter of 2015. The F₆ generation was planted in Shanghai in the summer of 2016, and molecular markers were used to verify the purity of the three resistant genes i.e., *Pi9*, *Pikh*, and *Pi5*. Besides, 8 lines were selected via comprehensive trait inspection. The F₇ generation was planted in Hainan during the winter of 2016, and 3 lines were selected through detection and matching. In the summer of 2017, lines with the best comprehensive agronomic traits and the strongest heterosis and numbered as 17S2174 were selected in Shanghai. The line growth was continued and seeds were propagated in Hainan during the winter of 2017, adhering to the technical appraisal in Shanghai in August 2018 and named Huhan 74S (Fig. 1).

Resistance identification

Blast resistance screening of the novel PTGMS line Huhan 74S harboring the three resistance genes was evaluated via artificial inoculation of the fungus onto Huhan 1S and Huhan 74S. The identification outcomes of disease resistance showed that Huhan 1S was resistant to 5 out of 14 blast strains, and 9 were susceptible with a resistance frequency of 35.7%. On the other hand, Huhan 74S was resistant to 12 strains with a resistance frequency of 85.7%, and the resistance spectrum was significantly enhanced compared to Huhan 1S (Table 1).

Strain	Resistance evaluation	
	Huhan 74S	Huhan 1S
A1	MS	S
A33	R	S
A49	R	MR
B1	MS	S
B13	R	S
B15	R	S
B5	R	MR
C13	R	R
D1	R	MS
D3	R	R
E1	R	MS
E3	R	MS
F1	R	S
G1	R	R
Resistance frequency (%)	85.7	35.7

To further determine the field resistance of the newly bred PTGMS line, natural induction identification was conducted at the blast epidemic area in Jinggangshan, Jiangxi Province. This was the experimental area for the identification of blast resistance of national rice regional experimental varieties. The results revealed that the Huhan 74S line was resistant to blast, with a score of 3 for leaf blast and 1 for neck blast. However, Huhan 1S was susceptible to both leaf and neck blast (Table 2).

Lines	Score of leaf blast	Score of neck blast	Resistance evaluation
Huhan 74S	3	1	R
Huhan 1S	7	7	S

The bidirectional selection is applied to obtain both high yield potential and good drought resistance in WDR. To clarify the drought resistance of the newly bred PTGMS line, we evaluated the drought resistance of Huhan 74S according to the agricultural industry-standard (NY/T 2863-2015). The drought resistance index and score of Huhan 74S were 0.98 and 2, respectively, which was similar to the resistance level of Huhan 1S (Table 3).

Lines	Drought Resistance Index	Score	Resistance evaluation
Huhan 74S	0.98	2	R
Huhan 1S	1.10	2	R
Hanyou 73CK	1.00	2	R
IR36CK	0.48	4	MS

The above resistance identification outcomes indicated that the novel PTGMS line Huhan 74S, which aggregated three broad-spectrum blast resistance genes by MAS, retained the strong drought resistance of the recipient parents. Besides, it showed significantly enhanced resistance to rice blast.

Fertility-sterility alteration pattern in the field and main agronomic traits

During the summer of 2017 in Shanghai, Huhan 74S was sown by stages for fertility observation. After heading, pollen microscopic examination was performed each day, and bagged self-pollination was simultaneously carried out. The results identified that the earliest heading date of sowing by stages was July 19th. Between July 19th and September 15th, daily pollen microscopy outcomes showed no pollen, implying high abortion. Between September 15th and 20th, the pollen abortion type was typical abortion, with a small amount of round abortion. After September 21st, the pollen gradually normalized. Between July 19th and September 20th, the seed setting rate of bagged self-pollination was 0, and the stable sterile period lasted 59 days (Fig. 2).

Huhan 74S has compact in plant shape, with strong stem and tillering ability, panicle type is medium, and uniform grain setting. It was sown in Shanghai on May 15th, 2017, and headed on August 5th (82 days), 81.3cm of plant height, 11.7 effective panicles per plant, 24.2cm of panicle length, 202.2 spikelets per panicle, 77.2% of stigma exposure rate in sterile phase. In Hainan, it was sown on November 8th, 2017, and headed on February 29th, 2018, (101 days), 86.2% of the seed setting rate, and 23.7g 1000 grain weight. Rice quality analysis based on the standards issued by the ministry showed that Huhan 74S was grade 3 (Table 4).

Traits	Sterile phase in summer season of 2017 in Shanghai	Fertile phase in winter season of 2017-2018 in Hainan
Duration from sowing to heading (d)	82.3	101.5
Plant height (cm)	81.3	80.1
No. of panicles per plant	11.7	15.2
Panicle length (cm)	24.2	23.3
No. of Spikelets per panicle	202.2	189.3
Seed setting rate (%)	—	86.3
1000-grain weight (g)	—	23.7
Stigma exertion percentage (%)	77.2	—
Brown rice percentage (%)	—	79.3
Milled rice percentage (%)	—	62.8
Head rice percentage (%)	—	68.7
Chalky rice percentage (%)	—	13.1
Chalkiness degree	—	3.9
Grain length (mm)	—	6.8
Grain length/width ratio	—	3.3
Alkali spreading value	—	6.0
Amylose content (%)	—	14.3
Gel consistency (mm)	—	65.4

Characteristics of fertility-sterility alteration in artificial climate chamber

The critical temperature point (CTP) of fertility-sterility alteration is of great practical significance for the safe seed production of the PTGMS line. Here, rice plants were placed in five artificial climate chambers and treated daily at mean temperature (DMT) of 21°C, 22°C, 23°C, 24°C, and 25°C for 12 consecutive days from the stage III of panicle differentiation. The pollen of the first five spikelets of each plant heading 5-16 days after the end of the environmental treatment in the control climate chamber was examined under a microscope. Consequently, the pollen of the newly bred PTGMS line Huhan 74S was completely sterile in the growth chamber with a DMT temperature of 23°C - 25°C (the pollen sterility rate of more than 99.5%). However, it was partially fertile at 21°C to 22°C, and this was consistent with the fertility alteration under the field natural conditions (Table 5, Fig. 2). Therefore, it was safe to conduct two-line hybrid seed production under conditions of DMT higher than 23°C.

Lines	Pollen sterility (%)				
	21°C	22°C	23°C	24°C	25°C
Huhan 74S	60.5	98.6	100	100	100
Huhan 1S	58.5	98.2	100	100	100

Agronomic traits and resistance performance of derived hybrids

Five hybrid rice combinations were derived from the new TGMS line Huhan 74S and its pollen parents, Huazhan, Chenghui 727, Hanhui No.3, Hanhui 808, Hanhui 8228. As a result, all combinations demonstrated satisfactory luxuriance and strong lodging resistance, with the yield potential reaching 9 -10 t/hm² (Table 6). This was equivalent to the control Fengliangyou No.4. The score of leaf blast in all combinations ranged from 1-3, the score of neck blast ranged from 1-3, while drought resistance level was above medium. Additionally, the drought resistance of the other four hybrid combinations was above medium resistance except that of Huhan74s / Chenghui727 which was drought-sensitive (Table 6).

Combinations	Growth duration (d)	Plant height (cm)	Panicle length (cm)	No. of panicles per plant	No. of grains per panicle	Seed setting rate (%)	1000-grain weight (g)	Grain yield (t/ha)	Blast resistance			Drought Score
									Score of leaf blast	Score of neck blast	evaluation	
Huhan 74S/Huazhan	114.0	107.7	23.2	14.2	186.5	83.0	22.9	9.58	1	1	R	3
Huhan 74S/Chenghui 727	118.0	112.3	26.1	14.5	169.5	87.8	24.2	9.30	2	3	MR	4
Huhan 74S/Hanhui 3	115.0	116.7	24.3	11.4	132.2	88.8	25.3	9.29	3	3	MR	2
Huhan 74S/Hanhui 808	118.0	108.0	24.5	11.7	187.0	87.2	25.6	9.68	2	1	R	2
Huhan 74S/Hanhui 8228	123.0	110.3	22.9	13.6	121.2	91.3	25.1	10.32	3	3	MR	2
Fengliangyou 4 (CK)	128.0	124.3	24.7	13.1	154.2	90.0	27.2	9.50	8	7	S	5

Discussion

In contrast with three-line hybrid rice, the two-line hybrid rice breeding system based on PTGMS line has advantages, including; (1) Male sterility is controlled by recessive nuclear gene, any genotype with good combining ability is used as the male parent, thus a wide selection range of restorer line. Also, the probability of obtaining heterosis by test crossing is higher than that by three-line method; (2) the PTGMS line remains unaffected by the relationship between restoration and maintenance, this is specifically suitable for the breeding of intersubspecific hybrid (Indica/japonica); (3) Hybrid seed production has a lower cost than that of three-line seed production, and the procedure of hybrid seed production is simplified by dual-use of one line. As an effective method of enhancing rice yield and quality, two-line hybrid breeding is widely used in China (Yang et al, 2007; Yuan, 2017). Nevertheless, the productivity of the two-line hybrid rice is severely limited by diseases, among which blast is considered the most destructive, causing huge yield losses (Ashkani et al, 2016). Molecular marker-assisted selection (MAS) is an important approach for disease resistance breeding; particularly, the use of cloned genes significantly reduces the breeding time and costs (Ishihara et al, 2014; Jiang et al, 2012). At present, the improvement of blast resistance of two-line male sterile lines is primarily based on marker-assisted selection of a single or two genes. Jiang et al (2015a) introduced the broad-spectrum and long-lasting rice blast resistance gene (*Pi2*) into the excellent Thermosensitive Genetic Male Sterile line (TGMS) C815S and selected four TGMS lines with blast resistance using dual selection approach of combining phenotypic and genotype selection with background selection. Based on Guangzhan 63-4S, Jiang et al (2015b) further bred a two-line sterile line with two resistant genes (*Pi2* and *Xa23*) using molecular markers. Yang et al (2019) effectively introduced blast resistance gene *Pi2* into PTGMS line Feng39S by MAS, the resulting improved male sterile line and hybrid combination significantly enhanced the blast resistance. In the practice of crop disease resistance breeding, the repeated use of a single gene for a long time easily causes the loss of resistance. Multigene pyramiding is conducive to broadening the resistance spectrum and enhances the resistance of crops. Resistance gene pyramiding breeding prevents the production risk caused by loss of resistance in varieties with a single resistance gene. Huhan 1S is a WDR PTGMS line with strong drought resistance, however, its application has been limited due to its poor blast resistance. This study introduced three blast resistance genes i.e., *Pi9*, *Pikh*, and *Pi5* into Huhan 1S through MAS and phenotypic selection, and a new PTGMS line Huhan 74S was bred. The polymerized blast resistance genes were broad-spectrum resistance genes at different loci, which were conducive for enhancing a stable and durable resistance of rice varieties. The results of artificial inoculation at the seedling stage and field naturally induced identification showed a significant increase in the blast resistance of Huhan 74S and its combinations.

Rice production is usually affected by biotic and abiotic stresses. Zhang (2007) introduced a concept of Green Super Rice (GSR), a development direction of rice in the future, aimed towards integrating all green traits into rice varieties to cope with the challenges of resources and the environment. Less pesticide, chemical fertilizers, water-saving and drought resistance, high quality, and high yield should be met during the production of GSR. Notably, WDR is a vital component of the GSR plan and its development changes the conventional rice planting mode to achieve resource-saving and environment-friendly (Luo, 2010). Drought resistance is a complex trait, affected by thousands of drought resistance genes and their interaction with the environment. The formation of drought resistance is caused by the interaction of multiple drought resistance genes, rather than a single drought resistance gene (Shinozaki and Yamaguchi, 2007; Hadiarto and Tran, 2011). Xia et al. (2019) proposed a model for adaptive differentiation between the upland and lowland rice during domestication. This model particularly in the evolution of drought resistance in the upland rice and its balance with productivity has been proven to be partially true in our efforts to develop WDR cultivars, where the bidirectional selection is applied to obtain both high yield potential and good drought resistance. Wei et al reported that under high-intensity stress selection in the target environment, several genes of conventional hybrid combinations in drought adaptation and drought resistance transcriptional regulatory network were retained and pyramided in the genome of breeding offspring. This significantly enhanced the drought resistance of Huhan 2B (Wei et al, 2016, 2017). In our study, we did not use the backcross breeding approach. In addition to the use of molecular markers to ensure the prospect selection of resistance genes, alternative screening of drought avoidance, drought tolerance, disease resistance, yield, and quality traits

were conducted in the segregated generation population. This was geared towards significantly retaining the characteristics of water-saving and drought resistance, high yield and quality traits were selected. The agronomic traits and drought resistance of the newly bred PTGMS line Huhan 74S were similar to those of Huhan 1S. Moreover, the disease resistance was significantly enhanced, thereby verifying the high efficacy of molecular markers combined with stress selection.

The critical temperature point (CTP) of fertility-sterility alteration of PTGMS line is the change of pollen from fertile to sterile or vice versa at a certain temperature. Whereas, a stable sterility duration (SSD) is the time (d) when pollen remains completely sterile at a specific place (Virmani et al, 2003). These two parameters are significant for the safe seed production of two-line hybrid rice. Here, the CTP of Huhan 74S was located at 23°C of DMT, and the SSD was longer (51 days). As such, Huhan 74S was used for seed production when the DMT was greater than 23°C at the booting stage and to breed PTGMS line below 23°C (Fig. 2, Table 5). The rice quality reached the Grade 3 high-quality rice standard issued by the Ministry, and the yield potential of the combination was high. Therefore, Huhan 74S passed the technical identification of Shanghai in August 2018 and has been incorporated with numerous breeding units in China, indicating a broad application prospect.

Conclusion

In this study, we employed molecular marker-assisted selection (MAS), conventional hybridization and high-intensity stress screening to integrate the broad-spectrum blast resistance genes *Pi9*, *Pikh*, and *Pi5* into Huhan 1S, an elite water-saving and drought-resistance rice PTGMS line, leading to the development of Huhan 74S. Huhan 74S and its derived hybrids showed resistance to rice blast and drought. Huhan 74S had a critical temperature point of fertility-sterility alteration and high rice quality. Thus, the newly developed PTGMS parental line Huhan 74S with *Pi9*, *Pikh*, and *Pi5* is currently being utilized for heterosis breeding of broad-spectrum blast-resistant two-line WDR hybrids and as an improved disease donor source for further PTGMS parental line improvement.

Materials And Methods

Plant materials

Huhan 1S, a WDR two-line male sterile line, was the recipient (male-sterile gene was derived from Y58S), while Huhan 1B (*Pi9*) and Huhan 91 (*Pikh* and *pi5*) were the donor parents of the resistance gene. Restorer lines (Huazhan, Chenghui 727, Hanhui No.3, Hanhui 808, Hanhui 8228) were used to identify the heterosis and evaluate the resistance of the novel male sterile line hybrids. The blast susceptible control was Lijiang Heituan Xingu, drought resistance control was Hanyou 73, drought-sensitive control was IR36, while the heterosis identification control was Fengliangyou No.4. All materials were provided by Shanghai Agrobiological Gene Center (SAGC).

DNA extraction and molecular marker detection

The genomic DNA of leaves was extracted from fresh young leaves at the peak tillering stage using the Cetyltrimethylammonium Bromide (CTAB) method (Murray and Thompson, 1980). The molecular marker *Pi9*-Pro closely linked to the target gene *Pi9* (Tian et al, 2016) (F: 5'-TGATTATGTTTTTATGTGGGG-3'; R: 5'-ATTAGTGAGATCCATTGTTCC-3'), the molecular marker FM143 closely linked to the target gene *Pikh* (Wang et al, 2014) (F: 5'-CCCAACATTGGTAGTAGTGC-3'; R: 5'-TCCTTCATACGCAACAATCT-3'), and the molecular marker M-*Pi5* closely linked to the target gene *Pi5* (Gao et al, 2010) (F: 5'-ATAGATCATGCGCCCTCTG; R: 5'-TCATACCCATTGGTCATT-3') were used for MAS. The primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. The PCR was performed in a 20- μ l reaction volume, including 2 μ l of genomic DNA (10ng/ μ l), 10 μ l of Taq PCR mastermix (Tiangen BioTech (Beijing) Co., Ltd.), 0.5 μ l of each primer (10 μ M), and 7 μ l of dd H₂O. The PCR procedure involved; pre-denaturation at 95 °C for 5 min, then 30 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, and extension at 72 °C for 5 min. Acrylamide gel electrophoresis of 6% was performed for PCR products, and silver staining detection was conducted based on previous methods (Creste et al, 2001).

Evaluation of rice blast resistance

In total, 14 main pathogenic strains (ZA1, ZA33, ZA49, ZB1, ZB5, ZB13, ZB15, ZC13, ZD1, ZD3, ZE1, ZE3, ZF1, and ZG1) in China were artificially inoculated and identified for blast resistance spectrum analysis. The strains were cultured in oat medium (oatmeal 30 g/L, tomato juice 150 mL/L, agar powder 20 g/L, pH 6.5) for 3-5 days at 28°C in the dark. Thereafter, they were cultured under constant light at 26 °C for 5-6 days to form conidia. The mature conidia were washed with sterile water to prepare an inoculum with a concentration of about 1×10^5 cfu/ml. The seedlings were inoculated with the prepared spore solution by artificial spray during the 3 leaves and 1 heart period. The inoculated strains were placed in a dark room at 25°C and 90% humidity for 24 h, then transferred into the growth chamber, to grow for 6 days at 25-28°C and 90% humidity under 12/12h (bright/ dark) photoperiod. The disease was investigated after 7d of inoculation. The natural disease nursery of rice blast was selected at Jinggangshan identification nursery in Jiangxi Province. The disease was graded based on the 0-9 grading standard of the International Rice Research Institute (IRRI) (IRRI 2002).

Evaluation of drought resistance

The experiment was conducted in the winter of 2017 at the experimental field of the Shanghai Agrobiological Gene Center in Lingshui County, Hainan Province. A total of two treatments, i.e., drought and control, were set for drought resistance evaluation. The test materials were repeated three times; nine rows were planted in each plot with nine plants in each row, i.e., a row spacing of 20 cm \times 23 cm. From seeding to stage II of panicle differentiation, intermittent irrigation was used to keep the field moist, without leaving any water layer. The panicles were treated with drought stress at stage II of differentiation. For drought-sensitive rice control varieties, the stress was halted when all leaf curling failed to recover for more than five days or the leaf dead rate reached 50% in the morning. Thereafter, the field water management was restored. The control treatment was set up in the adjacent paddy field, and the whole growth period

was as per conventional paddy field cultivation and management. The yield of plots was measured at the maturity stage and the drought resistance index was calculated (Makara et al, 2006).

Field fertility survey

The planting was executed in stages during the summer of 2017 at the Zhuanghang Experimental Station of the Shanghai Academy of Agricultural Sciences. From April 20th, the planting was conducted at intervals of every ten days, where 7 stages were planted. Single seedlings were planted in three rows, with a spacing of 20 cm × 16.7 cm between rows. In each row, 12 plants were planted. This experiment applied the conventional field water and fertilizer management. From the earliest panicle of the test materials, samples were collected after every 1 d for pollen microscopic examination to observe the pollen fertility and record the pollen sterility data and temperature. For each material, five panicles were collected. While selecting the spikelets for pollen microscopic examination, randomly bag an unflowered panicle of rice with a sulfuric acid paper bag on each plant to investigate the degree of self-sterility.

Agronomic traits and rice quality

The agronomic traits of the newly bred PTGMS line were investigated during the summer and winter of 2017 at Shanghai, and Hainan respectively. In the sterile phase at Shanghai, this study investigated duration (from sowing to heading), plant height, number of panicles per plant, panicle length, number of spikelets per panicle, and stigma exertion percentage. Two traits, the seed setting rate and 1000-grain weight were investigated in the fertile phase at Hainan. After storing the harvested and dried rice at room temperature for 3 months, the rice quality parameters were evaluated, including brown rice percentage, milled rice percentage, head rice percentage, chalky rice percentage, chalkiness degree (%), grain length (Mm), grain length/width ratio, alkali spreading value, amylose content (%) and gel consistency (Mm). The detection of various indicators was executed based on the Determination of Rice Quality (NYT83-2017).

Characterization of the newly bred PTGMS line for fertility-sterility alternation in growth chambers

In the summer of 2018, the newly bred PTGMS line was sown at the experimental field of Huazhong Agricultural University. Neat and healthy rice seedlings at the 5-leaf stage (about 25 days after sowing) were selected for transplantation, five plants were planted in each pot, and plastic labels were labeled to each plant. Five plant growth chambers (model: ZSX1500GS, Shanghai Jing Wins and Scientific Equipment Co., Ltd., China) were adjusted for trial operation a week before the actual use in the experiment. All the five plant growth chambers had 14 hours of light, a relative humidity level of 75%, and average daily temperatures of 21°C, 22°C, 23°C, 24°C, and 25°C. The rice material was placed in the plant growth chamber for 12 days of temperature treatment in the panicle differentiation stage III then moved to under natural conditions. The pollen grains of the first 5 spikelets of each plant that headed 5-16 days after the end of the treatment were observed under a microscope. Based on pollen morphological classification, the pollen grains were stained with I₂-KI, and the degree of pollen sterility per panicle was recorded (Virmani et al, 2003). Lines with an average pollen sterility rate above 99.5% were completely sterile.

Evaluation of derived hybrids

The novel PTGMS line was detected and matched with the restorer lines (Huazhan, Chenghui 727, Hanhui No.3, Hanhui 808, and Hanhui 8228). Meanwhile, the matched combination and the control Fengliangyou No.4 were detected in the summer of 2018 at the Zhuanghang Experimental Station of Shanghai Academy of Agricultural Sciences. The comparison experiment was arranged in random groups and repeated three times. Each plot was planted in 5 rows, with 12 plants in each row, and the row spacing of 20.0 cm × 16.7 cm. The entire growth period was recorded. After maturation, the middle 5 plants were collected to investigate the plant height, panicle length, number of panicles per plant, number of grains per panicle, seed setting rate, and 1000-grain weight. The plots were manually harvested and calculated in actual yield.

Abbreviations

PTGMS: Photoperiod and thermo-sensitive genic male sterile; CMS: Cytoplasmic male sterility; MAS: Marker-assisted selection; WDR: Water-saving and drought resistance rice; GSR: Green Super Rice; CTP: Critical temperature point; DMT: Daily mean temperature.

Declarations

Ethics approval and consent to participate

This study complied with the ethical standards of China, where this research work was carried out.

Consent for publication

All authors are consent for publication.

Availability of data and materials

All relevant data are provided as tables within the paper in the additional files.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

Y L, FY Z, LJ L and XQ Y conceived and designed the experiments. DY K, XX L, AN Z, FM W, ZQ P, JH W and JG B performed the experiments. Y L, FY Z, GL L and XQ Y analyzed the data. Y L wrote the paper. All authors read and approved the final manuscript.

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Figures

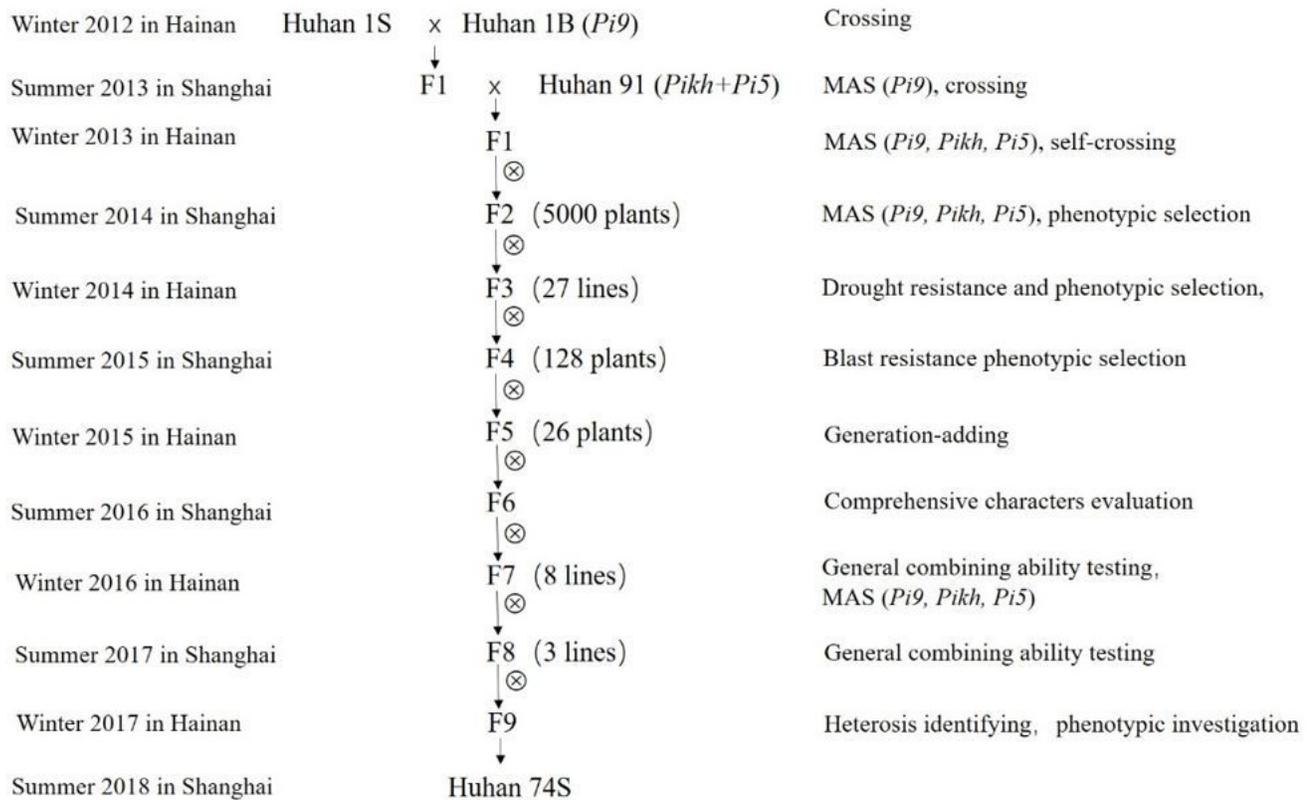


Figure 1

Breeding process of Huhan 74S

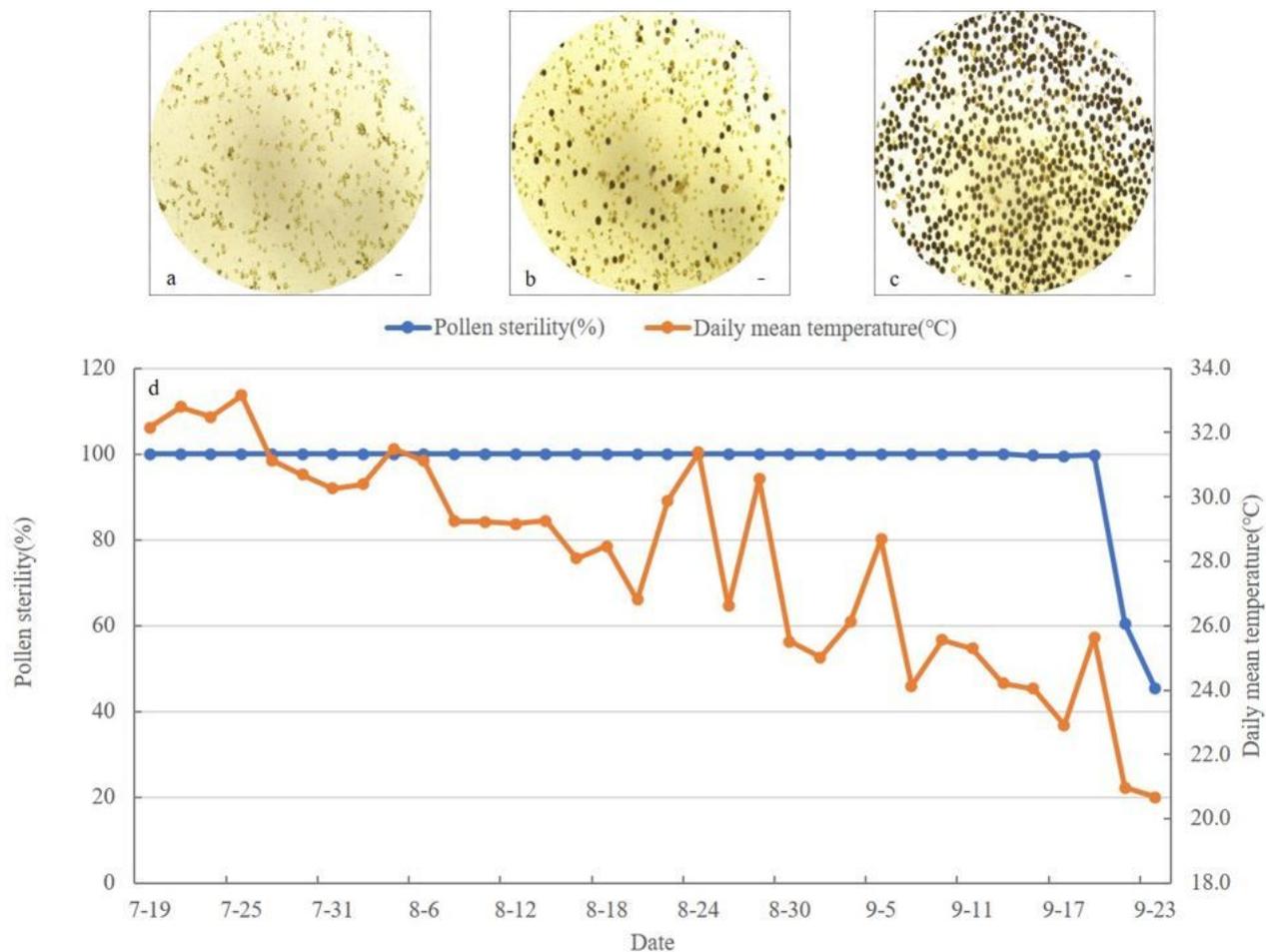


Figure 2
 Fertility-sterility alteration pattern of Huhan 74S in 2017 at Shanghai a-c Pollen grains from Huhan 74S on 12th August (a), 21st September (b) and 23th September (c). d Dynamic pollen sterility expressions of Huhan 74S relative to daily mean temperature (DMT) data between 19th July to 23th September in 2017 at Shanghai. Scale bars: 50 μ m