Development of Rice Variety with Durable and Broad-Spectrum Resistance to Blast Disease Through Marker-Assisted Introduction of *Pigm* Gene

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Research Article

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Abstract

Rice blast, caused by *Magnaporthe oryzae* (*M. oryzae*), is one of the most destructive diseases threatening rice production worldwide. Development of resistant cultivar using broad-spectrum resistance (*R*) gene with high breeding value is the most effective and economical approach to control this disease. In this study, the breeding potential of *Pigm* gene in *geng/japonica* rice breeding practice in Jiangsu province was comprehensively evaluated. Through backcross and marker-assisted selection (MAS), the *Pigm* was introduced into two *geng* rice cultivars (Wuyungeng 32/WYG32 and Huageng 8/HG8). In each genetic background, 5 advanced backcross lines with *Pigm* (ABLs) and same genotypes as the respective recurrent parent in the other 13 known *R* gene loci were developed. Compared with the corresponding recurrent parent, these ABLs all exhibited stronger resistance in seedling inoculation assay using 184 isolates collected from most rice growing regions of the lower region of the Yangtze River. With respect to panicle blast resistance, all ABLs reached high resistant level to blast disease in three consecutive years' tests with the inoculation of total 7 mixed-conidial suspensions collected from different regions of Jiangsu province. In natural field nursery assays, the ABLs showed significantly higher resistance than the recurrent parents. No common change on importantly morphological traits and yield-associated components was found among the ABLs, demonstrating the introduction of *Pigm* had no tightly-linked undesirable effect on rice economically important traits. Notably, one rice line with *Pigm*, designated as Yangnonggeng3091, had been authorized as a new variety in Jiangsu province in 2021, showing excellent performance on both grain yield and quality as well as the blast resistance. Together, these results suggest that the *Pigm* gene has a high breeding value in developing rice varieties with durable and broad-spectrum resistance to blast disease.

Background

Rice blast, caused by the fungus *Magnaporthe oryzae* (*M. oryzae*), is one of the most devastating diseases of rice worldwide (Dean et al. 2012). The yield loss caused by rice blast was 10%-30% in epidemic years and reached 40%-50% or even higher in serious cases (Skamnioti and Gurr 2009; Li et al. 2019). For example, it brought approximately 566 thousand tons of yield loss in 2015 alone in China (Liu et al. 2015). Developing resistant cultivars by marker assisted selection (MAS) of resistance genes has been proven to be the most effective, economical and eco-friendly strategy to control blast disease (Xiao et al. 2020).

To date, more than 350 quantitative resistance loci (*QRLs*) and 100 qualitative resistance (*R*) genes conferring resistance to *M. oryzae* have been identified, of which at least 32 *R* genes and 5 *QRL* genes have been cloned (Li et al. 2017; Yin et al. 2021). Comparatively, *QRL* genes, like *Pi21* and *bsr-d1*, generally have broad-spectrum and durable resistance because they mostly not belong to 'gene for gene' resistance and not exert strong selection pressure on pathogen. Through genome editing, Zhou et al. (2021) and Tao et al. (2021) obtained some lines that contained either of the two genes, *Pi21* and *bsr-d1*, and both. After inoculation, they found that the lines contained both *QRL* genes showed apparently stronger resistance than the lines possessed one of them, implying the pyramiding of *QRL* genes is a feasible approach to develop broad spectrum resistance variety. However, due to relatively small effects of *QRL* genes on resistance especially on panicle resistance as well as limited number of *QRL* genes isolated so far, deployment of *R* genes with broad-spectrum resistance is of particular importance in current rice breeding project. To date, only a few *R* genes were considered conferring broad-spectrum resistance, such as *Piz*, *Piz-t*, *Pi1*, *Pi2*, *Pi9*, *Pi33*, *Pi54*, *Pigm* and *Pi40* (Xiao et al. 2020; Li et al. 2020). Among them, *Pigm*, isolated from a Chinese local variety Gumei 4 with broad-spectrum and durable resistance to blast, exhibited resistance to 50 isolates of *M. oryzae* from all over the world (Deng et al. 2006 and 2017). It is particularly interesting since it contains a pair of antagonistic genes, *PigmR* and *PigmS*. *PigmR* gives rice broad-spectrum resistance, but it reduces rice 1000-seed weight and lastly affects grain yield. *PigmS*, in contrast, offset the adverse effect of *PigmR* on yield through improving the seed setting. More importantly, *PigmS* inhibits *PigmR* mediated broad-spectrum disease resistance through competing with *PigmR* to form a heterodimer, which alleviates the

selection pressure of the pathogenic evolution of *M. oryzae* races and so make *PigmR* has durable resistance (Deng et al. 2017), implying its importance in rice breeding program.

Leaf blast and panicle blast are two major types of rice blast diseases, and panicle blast is more importance on yield loss because it occurs on panicle neck directly restricting grain filling (Titone et al. 2015). Resistance to panicle blast and leaf blast at seedling stage is often inconsistent, which means that the resistance of some *R* genes are not effective across the whole development stages (Puri et al. 2009; Ishihara et al. 2014; Liu et al. 2016). Therefore, it is particularly important to employ broad-spectrum *R* genes independent of development stage in rice molecular breeding. However, most studies focused on seedling blast resistance rather than panicle blast resistance, because the panicle inoculation generally needs large space, and is time-consuming and easily affected by environment compared to spraying inoculation at the seedling stage (Hao et al. 2011; Xiao et al. 2020). Most of the broad-spectrum *R* genes mentioned above have not been evaluated for panicle blast resistance. Recently, a set of near-isogenic lines carrying six resistance alleles (*Piz*, *Piz-t*, *Pi2*, *Pi9*, *Pi40* and *Pigm*) at the *Piz* locus, respectively, under the background of *xian/indica* rice variety Yangdao 6 and *geng/japonica* rice line 07GY31, were developed by Wu et al. After inoculation with *M. oryzae* isolates from different ecological regions of China, they found that *Pigm* showed more broad-spectrum resistance to both seedling blast and panicle blast than the others *R* genes (Wu et al. 2016 and 2017).

Geng rice is an important food source in China, and more than 85% of rice area are *geng* rice in Jiangsu province (Zhang et al. 2011). Recently, the occurrence of rice blast especially for panicle blast have presented an increasingly serious threat on rice production in Jiangsu province. For instance, around 70% of rice fields were destroyed by panicle blast in 2014, resulting in huge yield losses (He et al. 2015; Wei and Song 2014). Studies have shown that some *R* genes, like *Pib*, *Pita*, *Pish*, *Pik-h*, *Piz* and *Pi54*, have been more widely used in *geng* rice varieties authorized in Jiangsu province (Fan et al. 2014; Wu et al. 2015; Wang et al. 2020), while, with the genetic variation of *M. oryzae* strains, these *R* genes were gradually losing their resistance (Zhu et al. 2018; Wang et al. 2020). For the *R* gene *Pigm*, however, to the best of our knowledge, almost no *geng* varieties currently released in Jiangsu province contain this gene. Recently, Zeng et al. (2018) introduced *Pigm* into three different *geng* rice varieties through MAS, and found that the BC₄F₁ plants with heterozygous *Pigm* allele showed strong panicle blast resistance against *M. oryzae* races from southern China. In addition, Wang et al. (2019) and Chen et al. (2020) employed two different *geng* rice varieties from Jiangsu province to cross or backcross with Gumei 4, and found that the plants carrying *Pigm* gene all exhibited at least moderate resistance to panicle blast. These studies suggest that *Pigm* has a breeding potential in improving blast resistance of *geng* rice in Jiangsu province. However, due to the fact that *Pigm* locus is from a landrace Gumei 4 and the adverse effect of *PigmR* on 1000-seed weight reported by Deng et al. (2017), the breeding value of *Pigm* locus in *gene* rice breeding project in Jiangsu province remains to be extensively evaluated.

In this study, we introduced *Pigm* gene into two high yield and good quality *geng* rice cultivars in Jiangsu by MAS, and obtained a series of advanced backcrossing lines (ABLs) with *Pigm* gene and excellent performance in field. Furthermore, we comprehensively evaluated the resistance of the ABLs to both seedling and panicle blast diseases using 184 *M. oryzae* isolates collected from multiple places in the lower region of Yangtze River and especially in Jiangsu province. In addition, no tightly-linked undesirable effects on importantly economical and agronomical traits were found in the introduction of *Pigm*. Particularly, one of the advanced lines has been authorized as a new variety in Jiangsu province, which showed excellent blast resistance as well as high yield and good quality. Together, our data infer that *Pigm* gene has a high breeding value in *geng* rice breeding project towards broad-spectrum and durable blast resistance and the resistant ABLs generated in this study are of high valuable in breeding practice in future.

Results

Development of ABLs by marker-assisted selection

To determine the breeding potential of *Pigm* gene utilized in geng rice breeding program in Jiangsu province, we introduced *Pigm* into two well-known *geng* rice cultivars WYG32 and HG8 by MAS, respectively (Fig. 1). In BC₄F₅ generations, we obtained 13 and 16 ABLs, which contained homozygous *Pigm* gene and shared similar agronomic traits with the recurrent parents WYG32 and HG8, respectively. These lines were named WYG32-PG-1 to WYG32-PG-13 and HG8-PG-1 to HG8-PG-16 (Additional file 1: Table S1).

In order to exclude the influence of other rice blast *R* genes, we further detected the genotypes of these ABLs in other 13 known *R* gene loci (*Pia*, *Pib*, *Pid2*, *Pid3*, *Pik*, *Pikm*, *Pikh*, *Pit*, *Pita*, *Pi1*, *Pi5*, *Pi25* and *Pb1*) using functional markers. We found that 11 of the 13 and 12 of the 16 ABLs carried same *R* gene combination as the recurrent parents WYG32 and HG8, respectively; and among them, 5 ABLs were selected from each background for subsequently resistant evaluation and agronomic traits assessment (Additional file 1: Table S1).

ABLs showed more broad-spectrum resistance at the seedling stage to *M. oryzae* strains collected from the lower region of the Yangtze River

To evaluate the resistance effect of *Pigm* gene, total of 144 *M.oryzae* isolates were firstly used in inoculation assay. Among them, 84 were from all most all cities in Jiangsu province, and the remaining isolates were from major rice growing provinces in the lower region of the Yangtze River (Fig. 2A). Results showed that the susceptible variety LTH was susceptible to all 144 isolates, with resistance frequencies of 0 (Fig. 2B), indicating that our inoculation assays were successful. The resistance frequencies of the recurrent parents WYG32 and HG8 were 71.53% and 75.46%, respectively, while the ABLs were found resistant to almost all isolates with resistance frequencies ranging from 98.61–100% (Fig. 2B). This indicates the *Pigm* has broad spectrum resistance to *M.oryzae* isolates from the lower region of the Yangtze River.

To further determine the potential of *Pigm* in current rice breeding program in Jiangsu province, we then collected 85 isolates in 2019 from the locations that have been previously collected. Through functional marker of the 10 known avirulence gene loci (*Avr1-Co39*, *Avri-Pita*, *ACE1*, *Avr-Pib*, *PWL 1*, *Avr-Pia*, *Avr-Pi9*, *Avr-Pizt*, *Avr-Pik* and *Avr-Pii*), we found that these isolates had significant variation at the 10 know avirulence gene loci (Additional file 2: Table S2). Based on the phylogenetic result using genotyping data at these avirulence gene loci, we could classified these 85 isolates into 4 groups (RA, RB, RC, and RD) (Additional file 3: Table S3). After combining the location information, we found that each of the 4 groups contained the isolates from all different ecological regions, implying that the genetic variation of these isolates was not caused by the ecological regions. Then, in each group, 10 isolates from different locations were selected and used in seedling blast inoculation assay. We found that all ABLs showed high resistance to these isolates with resistant spectrum from 98.33–100%, which significantly higher than the corresponding recurrent parent and the susceptible control LTH with resistant spectrum of 0% (Fig. 2B, C). Taken together, these data demonstrate that introducing *Pigm* into *geng* rice varieties in Jiangsu province is able to greatly broaden their resistance spectrum to blast disease, indicating a great potential of *Pigm* in rice breeding program especially in Jiangsu province.

Abls Exhibited Durable And High Resistance Against Panicle Blast

To further assess the panicle blast resistance of these ABLs, total of 7 mixed conidial suspensions of the isolates collected from Jiangsu were used to inoculate ABLs at the booting stage. The mixed conidial suspensions of 2018, 2019 and 2020-1 were provided by Professor Yongfeng Liu, which were used in the region test of Jiangsu rice varieties for identifying resistant level of each candidate rice variety in 2018, 2019 and 2020, respectively. The other 4 mixed suspensions (2020-2, 2020-3, 2020-4 and 2020-5) were made by the present study with each comprised by 6 representative isolates that were selected from each of the above 4 groups (RA, RB, RC, and RD) based on genotypes and locations distribution (Fig. 3; Additional file 3: Table S3). In these 7 trials, the disease grades of the control WYG32 were

9 in 4 trials (2018, 2020-3, 2020-4 and 2020-5) and 7.4 ~ 8.6 in the other 3 trials (2019, 2020-1 and 2020-2), showing high susceptible to panicle blast. In contrast, all 5 ABLs in this background reached high resistant level to panicle blast with the disease grade ranged from 0 to 0.8. For another background, the disease grades of HG8 was in the range of 3 ~ 5 in all 7 trials, exhibiting moderate resistance to panicle blast. However, all 5 ABLs under HG8 background displayed high resistance to panicle blast with the disease grade ranged from 0 to 0.6. Taken together, these results indicated that transferring *Pigm* into *geng* rice varieties in Jiangsu province could greatly improve rice resistance to panicle blast.

Abls Displayed Well Performance In Blast Nursery Field

To evaluate the resistance effect of ABLs under natural conditions with high blast disease pressure, field assays were performed in Ganyu, Jiangsu province in 2018–2019. As shown in Fig. 4, the recurrent parent WYG32 showed moderate susceptibility in 2018 and high susceptibility in 2019 with diseased-panicle rates of 17.7% and 92.3%, respectively, indicating the severity of this region on blast disease. However, as expected, the diseased-panicle rates of all 5 ABLs under WYG32 background was 0% in both years, reaching high resistant level. For another background, the HG8 showed moderately resistant levels in two years with diseased-panicle rates of 7.8% in 2018 and 9.8% in 2019, whereas all the ABLs in this background showed high resistance in two years except the line HG8-PG-5 that reached resistant level (1.1%) in 2019. The results in the blast nursery further demonstrated that transferring *Pigm* into *geng* rice varieties in Jiangsu province could apparently enhance rice resistance to blast.

No apparently adverse effects were detected among ABLs compared with the recurrent parent

To check whether the introduction of *Pigm* could affect agronomic traits and grain quality, we measured these traits for ABLs and the recurrent parents. As listed in Table 1, we found that, in total, most ABLs displayed similar traits as the corresponding recurrent parent. A few traits were found significant difference between some lines and the control, while these were not common among different ABLs. For instance, ABLs WYG32-PG-4, WYG32-PG-8, WYG32-PG-11 and HG8-PG-6 showed higher plant height than the corresponding recurrent parents, while the others ABLs displayed closely plant height with the controls. This indicated that these changed traits were not closely linked with *Pigm*, implying that the introduction of *Pigm* has no tightly-linked adverse effects on rice agronomical traits and grain quality, at least for the varieties tested in the our study.

A new rice variety Yangnonggeng 3091 was authorized with strong blast resistance, high grain yield and good quality

Besides of the MAS selection of *Pigm*, we also conducted agronomic trait selection and evaluation, and which allowed us to obtain a new rice line in BC₃F₆ generation under HG8 background, designated as Yangnonggeng 3091 (YNG3091, Fig. 5A). In terms of panicle blast resistance, YNG3091 exhibited high resistance (disease grade, 0 ~ 0.4) to all five mixed conidial suspensions (2020-1, 2020-2, 2020-3, 2020-4 and 2020-5), while the well-known control variety Huaidao 5 (HD5) in rice region test of Jiangsu province showed moderately susceptible level (disease grade, 5.4 ~ 7) (Fig. 5B). Moreover, YNG3091 recorded high resistance level in natural blast disease nurseries in 9 different cities of Jiangsu province (Fig. 5B). For grain yield, we found that YNG3091 increased by 4.4% per 667 m² compared with HD5 (Fig. 5C). In addition, the YNG3091 showed slightly higher plant height than HD5 but having closely total growth period. With respect to grain quality, we found that the chalkiness rate and chalkiness degree of YNG3091 were significantly lower than those of HD5, and the other traits were all similar with those of HD5 (Fig. 5D). Due to good performance on grain appearance, YNG3091 was evaluated as grade II of good quality rice according to the rice quality standard of China's agricultural industry (MARAPRC 2002). In 2021, the YNG3091 was authorized as a new rice variety (Approval No.: Sushengdao 20210046) in Jiangsu province (Fig. 5). This further confirmed that *Pigm* gene has great potential in developing new rice varieties with high blast resistance.

Table 1
Performance of basic agronomic and grain quality traits in ABLs and the recurrent parents.

Cultivars/lines	Plant height (cm)	Heading date (d)	Panicle number	Panicle length (cm)	Grain number per panicle	1000-seed weight (g)	Seed setting rate (%)	Chalkiness rate (%)	Amylose content (%)
WYG32	98.06 ± 2.23	98.0 ± 0.71	12.6 ± 0.55	17.94 ± 1.18	131.8 ± 5.63	26.85 ± 0.43	93.65 ± 1.01	15.08 ± 1.16	15.76 ± 0.59
WYG32-PG-4	102.48 ± 2.69**	99.2 ± 0.84*	13.2 ± 0.84	18.14 ± 1.23	132.6 ± 4.16	26.93 ± 0.44	93.10 ± 1.74	14.56 ± 0.81	15.28 ± 0.55
WYG32-PG-5	99.76 ± 2.10	97.4 ± 0.55	13.0 ± 0.71	18.64 ± 1.17	133.2 ± 4.15	26.96 ± 0.36	92.45 ± 1.55	15.30 ± 0.93	16.36 ± 0.87
WYG32-PG-8	103.94 ± 2.10**	97.2 ± 0.45	12.4 ± 1.14	17.86 ± 1.19	130.6 ± 3.97	26.79 ± 0.54	95.59 ± 2.16	15.64 ± 0.84	15.82 ± 0.98
WYG32-PG-11	104.74 ± 1.83**	100.2 ± 0.84**	13.6 ± 1.14	18.12 ± 0.95	131.2 ± 4.32	26.86 ± 0.39	95.49 ± 1.58	13.88 ± 0.56*	15.54 ± 0.55
WYG32-PG-12	99.42 ± 1.45	96.2 ± 0.84**	13.4 ± 0.89	17.62 ± 1.03	130.8 ± 3.70	26.76 ± 0.28	93.69 ± 2.10	14.28 ± 0.76	16.52 ± 1.00
HG8	100.80 ± 3.50	97.4 ± 1.14	14.2 ± 1.30	18.38 ± 0.90	136.0 ± 5.24	27.56 ± 0.37	92.41 ± 1.76	17.82 ± 0.76	19.18 ± 1.01
HG8-PG-2	101.28 ± 2.19	101.0 ± 0.71**	13.6 ± 0.55	18.86 ± 1.06	141.8 ± 4.82*	27.13 ± 0.33	93.40 ± 2.19	10.38 ± 1.36**	16.06 ± 0.72**
HG8-PG-4	102.34 ± 2.00	100.2 ± 0.84**	13.8 ± 0.84	19.04 ± 1.18	143.2 ± 4.09**	27.06 ± 0.39*	93.62 ± 1.52	11.22 ± 1.16**	16.40 ± 0.89**
HG8-PG-5	100.42 ± 1.83	95.4 ± 1.14**	14.4 ± 0.55	18.58 ± 1.42	138.2 ± 3.03	27.39 ± 0.48	95.51 ± 1.78*	16.94 ± 1.83	18.58 ± 0.90
HG8-PG-6	104.28 ± 2.43*	96.2 ± 1.10	13.6 ± 1.34	18.74 ± 1.15	140.8 ± 4.60	27.14 ± 0.29	92.04 ± 1.20	15.80 ± 2.13	17.38 ± 0.69**
HG8-PG-16	102.98 ± 1.51	97.8 ± 0.84	13.8 ± 0.84	18.48 ± 1.13	137.2 ± 3.83	27.51 ± 0.27	92.11 ± 3.19	18.92 ± 1.81	19.02 ± 0.92
* $P < 0.05$, ** $P < 0.01$ compared with the corresponding respective recurrent parent by Student's t-test.									

Discussion

Pigm conferred durable and broad-spectrum resistance to both seedling and panicle blast

Deployment of broad-spectrum and durable resistance gene is of great importance to develop broad-spectrum resistance variety. In this study, we developed several ABLs contained *Pigm* gene in genetic backgrounds of two different *geng* rice varieties from Jiangsu province by MAS (Fig. 1). Wang et al. (2020) found that besides of *Pigm* locus, 13 known *R* genes also distributed in *geng* rice varieties of Jiangsu province with various distribution degree. A few *R* genes and *R* gene combinations showed apparently different contributions on rice blast resistance. Therefore, in order to reduce the effects from the other *R* genes on the evaluation of *Pigm* resistance, we selected 5 ABLs in each background that showed same genotypes with the corresponding recurrent parent in the other 13 known *R* gene loci (Additional file 1: Table S1). After

inoculation with 184 *M.oryzae* isolates in seedling stage, we found that all ABLs showed significantly more broad-spectrum resistance than the corresponding recurrent parent. These isolates collected from different locations in the lower region of the Yangtze River especially in Jiangsu province, displaying a wide representative (Fig. 2). It is expected that after years of different varieties planted, the major epidemic isolates in field might be changed. For further testing the resistance effect of *Pigm* on latest *M.oryzae* isolates, 40 of the 184 isolates were collected in 2019 from the locations that previously collected in 2014, which showed an apparently genetic diversity based on the genotype analysis of known avirulence gene loci (Additional file 2, 3: Table S2, 3). After combining the other studies using different isolates from at least 3 different rice growing ecological regions (Tian et al. 2016; Dai et al. 2018; Zhou et al. 2021), we infer that *Pigm* gene confers broad-spectrum resistance to rice blast.

In terms of breeding practice, it is of great significance to regularly collect latest epidemic isolates to evaluate the resistance of rice lines to rice blast, especially panicle blast. The lab of Professor Yongfeng Liu (Plant Protection Institute of Jiangsu Academy of Agricultural Sciences) has been performed the evaluation experiment of blast resistance for the rice lines in the region test of rice new varieties in Jiangsu province. Therefore, they have to isolate large number of *M.oryzae* isolates from all over the province each year, and through pathogenicity test, 6 of the isolates showing strongest pathogenicity and genetic diversity were then used for panicle inoculation next year (private communication with Prof. Liu). The mixed conidial suspensions, named 2018, 2019 and 2020-1, were from Liu's lab and we found that the ABLs showed high resistance level to all these conidial suspensions in panicle blast inoculation assay. Similarly, in the inoculation assay using the other 4 mixed conidial suspensions, the ABLs reached high resistance levels either (Fig. 3; Additional file 2, 3: Table S2, 3). Meanwhile, the results of natural evaluation in blast nursery in Ganyu in consecutive 2 years (2018–2019) and 9 different cities in 2020 showed a consistent trend with artificial inoculation (Fig. 4, 5B). These results indicate that *Pigm* gene has strong effects on improving rice panicle blast resistance, at least for *geng* rice varieties in Jiangsu province. Collectively, although the fact that durable resistance of *Pigm* is still required to be monitored after numbers of rice varieties with *Pigm* gene widely planted, the *Pigm* gene could be considered conferring durable resistance because it displayed very broad-spectrum resistance to both seedling and panicle blast.

The effect of *Pigm* gene on reduction of rice grain weight was not detected in present study

Linkage between *R* genes and deleterious alleles (Linkage drag) is one of the major reasons limits their utilization in breeding practice. Many broad-spectrum *R* genes, such as *Pi1*, *Pi9*, *Pi33*, *Pi40*, *Pi56* and *Pigm*, are mainly distributed in local special germplasms but not modern cultivars, and their cumbersome chain of linkage drag might produces negative effects on agronomic traits (Xiao et al. 2020). The *Pigm* gene was reported could achieve yield balance by reducing grain weight but increasing seed setting (Deng et al. 2017), while, decrease in grain weight is generally an undesirable trait in breeding practice. In the present study, we obtained five ABLs in each of the two recurrent parents. Notably, when compared with the corresponding recurrent parent, we found that all ABLs displayed almost same 1000-grain weight with the control except for one line HG8-PG-4 with significant lower 1000-grain weight than that of HG8 (Table 1). Grain weight is a typical of quantitative trait that controlled by multiple genes, and so far at least 44 genes have been confirmed to be involved in regulation of grain weight in rice (Li et al. 2018). Specially, wide interactions among different grain weight related genes and complex affection of hormone signaling on grain weight have been found (Zuo et al. 2014), which will undoubtedly affect the expression of minor effect of single gene in complex genetic background. In the breeding process for developing these ABLs, the plants without good performance on agronomic traits, including grain size, were in fact not selected. In addition, due to the fact that the backcross and self-pollinated populations in each generation not contained large number of plants (Fig. 1), so we inferred that the weak reduction of *PigmR* on grain weight could be easily eliminated by agronomical trait selection. This implies that *geng* varieties, or at least in the two varieties of this study, may contain gene(s) that interacts or complements with *PigmS* and ultimately offsets the effect of *PigmS* on grain size/weight, which is worthy of further investigation.

Notably, a rice line YNG3091 from BC₃F₆ generation, has been authorized as a new rice variety in Jiangsu province, which displays well resistance to both seedling blast and panicle blast as well as excellent performance on both grain yield and quality (Fig. 5). Most recently, a new *geng* variety Jinxiangyu 1 carried *Pigm* was also developed via MAS in Jiangsu province, which also showed excellent blast resistance and high yield (Xiao et al. 2021). Together, these demonstrate that *Pigm* has a high breeding value in developing blast resistant variety and the reduction effect of *PigmS* on grain weight could be overcome by agronomical trait selection. In addition, this study provides several new rice lines including the variety YNG3091 carried *Pigm*, which can be directly used as new donors in rice breeding project and ultimately speed up the development of blast resistant varieties in Jiangsu province and its surrounded region.

Conclusions

The *Pigm* gene has a high breeding value in developing rice varieties with durable and broad-spectrum resistance to blast disease and the resistant lines including the variety YNG3091 carried *Pigm* generated in this study are of high valuable in breeding practice in future.

Materials And Methods

Plant materials and pathogens

The *geng* rice line 07GY31-*Pigm*, which carries *Pigm* from Gumei 4 and exhibits broad-spectrum resistance against rice blast (Wu et al. 2017), was used as the donor of *Pigm*, and two *geng* rice cultivars, Wuyungeng 32 (WYG32) and Huageng 8 (HG8), were authorized by Jiangsu province, showing high yield and good grain quality, were selected as recurrent parents to develop blast resistance materials/varieties with high breeding potential.

144 and 85 *M. oryzae* isolates were collected and obtained from infected fields of 22 cities in the lower region of the Yangtze River (including Jiangsu, Anhui, Hubei, and Zhejiang provinces) in 2014 (Wu et al. 2017) and 13 different cities/counties in Jiangsu province in 2019, including Jintan (JT), Wujin (WJ), Zhenjiang (ZJ), Yangzhou (YZ), Baoying (BY), Jiangdu (JD), Gaoyou (GY), Taizhou (TZ), Huaian (HA), Siyang (SiY), Ganyu (GanY), Sheyang (SheY) and Yancheng (YC). The isolation and cultivation of single-spore strains, and inoculum preparation were carried out as described previously (Puri et al. 2009).

Molecular marker assay

Genomic DNA was extracted from one-month-old rice leaves and the mycelium of *M. oryzae* strains using the CTAB method (Zhang et al. 1992). The tightly linked molecular marker ZJ58.7 (2013-2018) and the functional marker GMR-3 (2019-2020) were selected to monitor *Pigm* (Yu et al. 2013; Wang et al. 2019). The functional markers used to detect 13 cloned blast R genes (*Pia*, *Pib*, *Pi-d2*, *Pi-d3*, *Pik*, *Pikm*, *Pik-h*, *Pit*, *Pi-ta*, *Pi1*, *Pi5*, *Pi25* and *Pb1*) in rice plants and 10 isolated avirulence genes (*Avr1-Co39*, *Avr1-Pita*, *ACE1*, *Avr-Pib*, *PWL1*, *Avr-Pia*, *Avr-Pi9*, *Avr-Pizt*, *Avr-Pik* and *Avr-Pii*) in *M. oryzae* strains referred to the primer sequences described by Wang et al. (2020) and Shen et al. (2019), respectively. The reaction mixture (20 µL) for PCR amplification consisted of 2 µL DNA template (about 40 ng µL⁻¹), 1 µL of each primer (10 µmol L⁻¹), 2 µL MgCl₂ (25 mmol L⁻¹), 0.4 µL dNTP (10 mmol L⁻¹), 2 µL 10×PCR buffer, 0.2 µL Taq polymerase enzyme (5 U µL⁻¹) and 11.4 µL ddH₂O. PCR amplification was carried out with the following protocol: a pre-denaturation for 5 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s at the annealing temperature, 1 min at 72°C, and a final extension 72°C for 10 min. The PCR products were separated by electrophoresis on 4% agarose gels with ethidium bromide and photographed using a gel imaging system (Tanon 2500).

MAS procedure for transferring *Pigm* and developing new lines

The MAS scheme for transferring *Pigm* into two genetic backgrounds is presented in Fig. 1. *Pigm* was monitored in progenies from either backcrossed or self-pollinated plants using the molecular markers. The donor of *Pigm* (07GY31-*Pigm*) was crossed with each of the two recurrent parents (WYG32 and HG8) to obtain F₁ plants in 2013. Then F₁ plants of each cross were backcrossed with the corresponding recurrent parent till the advanced 10 independent BC₄F₁ plants. In 10 BC₄F₂ populations, 5 plants per BC₄F₂ population (50 plants in total) with homozygous *Pigm* allele and good agronomic traits were screened for individually harvesting. In BC₄F₃ populations, total of 13 and 16 lines with similarly agronomic phenotype with WYG32 and HG8 were selected, respectively. In each BC₄F₃ line, only one plant with excellent performance through visible selection was kept, and the same selection strategy was used in each BC₄F₄ line. Lastly, till BC₄F₅, the agronomic characters of each lines presented uniformity and were harvested together as a pure line. All BC₄F₅ homozygous lines were then examined for other 13 cloned blast *R* genes, *Pia*, *Pib*, *Pi-d2*, *Pi-d3*, *Pik*, *Pikm*, *Pik-h*, *Pit*, *Pi-ta*, *Pi1*, *Pi5*, *Pi25* and *Pb1*, using functional markers. Finally, in each background, five BC₄F₅ ABLs with same *R* gene combination on these 13 *R* gene loci as the recurrent parents were selected in 2018 for subsequent experiments (Additional file 1: Table S1). Meanwhile, in the BC₃F₆ generation, excellent lines carrying *Pigm* were screened to participate in the region test of rice new varieties in Jiangsu province.

Evaluation for seedling blast resistance by artificial inoculation

Evaluation of seedling blast resistance by artificial inoculation was performed according to the method described by Wu et al. (2015) with slight modification in BC₄F₆ generation in 2019 and BC₄F₇ generation in 2020. 12 plants of each line/cultivar were grown in a plastic tray (60×30×4 cm) in the greenhouse maintained at 26 °C to 32 °C. The highly susceptible cultivar Lijangxintuanheigu (LTH) was used as the susceptible control. There were three replicate trays for each blast isolate. Three-week-old seedlings were placed in a glass box (70×45×33 cm) and were sprayed with 30 ml of an isolate conidial suspension using a vacuum pump (4-7.8 kg/cm²). The inoculated seedlings were cultured in the glass box maintained at 25°C in the dark for 24 h, and then were transferred to a transparent plastic box, covered with fresh-keeping film to maintain humidity, and grown under a 12 h light at 28 °C/12 h darkness at 25 °C cycle for 7 days. Disease reactions were recorded 7 days post-inoculation in accordance with standard procedures (Mackill and Bonman 1992). Resistance levels of tested materials were represented by resistance frequency, which was calculated as follows: number of incompatible isolates / total number of isolates inoculated × 100% (Wu et al. 2015).

Evaluation for panicle blast resistance by artificial inoculation and natural induction

The artificial identification of panicle blast resistance in field was performed as described previously (Wu et al. 2017) using mixed conidial suspension containing 6 representative *M. oryzae* isolates. A randomized complete block design was used with three biological replications. Each replication contained 10 rows and 10 plants per row, with row spacing of 11.1 by 30 cm. At the booting stage, 10 panicles were injected with 1 mL of each mixed conidial suspension (5×10⁴ conidia/mL). At the ripening stage, the panicle blast disease scores were evaluated according to a '0-9' rating method (MARAPRC 2014). According to disease scores, each rice line was then classified into differently resistant levels: highly resistant (0 ≤ disease grade < 1), resistant (1.0 ≤ disease grade < 3), moderately resistant (3.0 ≤ disease grade < 5.0), moderately susceptible (5.0 ≤ disease grade < 7.0), susceptible (7.0 ≤ disease grade < 9.0) and highly susceptible (disease grade = 9.0).

All tested materials were also subjected to the determination of blast resistance in blast nursery in the field of Ganyu county, Lianyungang city, Jiangsu, with high blast disease pressure in 2018-2019. A randomized complete block design was used with three biological replications. Each replication contained 10 rows and 10 plants per row, with row spacing of 11.1 by 30 cm. Resistance level in the blast nursery was evaluated based on the diseased-panicle rate which was calculated as the number of diseased panicles divided by the total number of panicles and multiplied by 100 (Ministry of

Agriculture and Rural Affairs of the People's Republic of China, 2014). According to the diseased-panicle rates, the tested materials with a diseased panicles rate of 0% were considered highly resistant, 0.1%-5% were resistant, 5.1%-10% were moderately resistant, 10.1%-25% were moderately susceptible, 25.1%-50% were susceptible, and 50.1%-100% were highly susceptible.

Examination of importantly agronomic and grain quality traits

The ABLs were planted in the field at Yangzhou University, Jiangsu, China. The planting method was the same as above (Evaluation for panicle blast resistance). Ten plants between the second and the ninth rows were randomly selected for measuring the importantly agronomic traits, which included plant height, heading date, panicle number, panicle length, grain number per panicle, 1000-seed weight, seed setting rate, and grain quality associated traits, including chalkiness rate and amylose content according to the previous description (IRRI 2002).

Data analysis

Data of blast disease severity and agronomic traits were sorted and plotted by Excel 2016. Significant differences between ABLs and the recurrent parents were analyzed using Student's *t*-test in the SPSS 19.0 program. The cluster analysis of 271 *M. oryzae* isolates was performed by using SPSS 19.0.

Abbreviations

M. oryzae

Magnaporthe oryzae

R

resistance

MAS

marker-assisted selection

WYG32

Wuyungeng 32

HG8

Huageng 8

ABLs

advanced backcross lines with *Pigm*

QRLs

quantitative resistance loci

YNG3091

Yangnonggeng 3091

HD5

Huaidao 5.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data supporting the conclusions of this article are provided within the article (and its Additional files).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SMZ, ZXC and ZMF designed the research. ZMF, MYL, XQW, KTW, JNW, ZWX, YYW, GDW, JHZ, KMH and YFZ performed the experiments and analyzed the data. ZMF wrote the manuscript. SMZ, ZXC, AHL and HQC revised the manuscript. All authors read and approved the manuscript.

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Figures

Figure 1

Marker-assisted selection (MAS) scheme for transferring *Pigm* and developing durable and broad-spectrum blast-resistance lines. WYG32, Wuyungeng 32; HG8, Huageng 8.

Figure 2

Blast resistance scores of ABLs and the recurrent parents at the seedling stage. **(A)** Distribution of 184 *M. oryzae* isolates used for seedling blast inoculation in the lower region of the Yangtze River. **(B)** Comparison of resistance frequencies among ABLs and the recurrent parents (WYG32 and HG8) against *M. oryzae* isolates. **(C)** The seedling blast disease reactions of ABLs against two *M. oryzae* isolates, 19-YZ-1 and 19-GanY-1. ** $P < 0.01$ compared with the recurrent parent by Student's t-test.

Figure 3

Panicle blast resistance scores of ABLs and the recurrent parents at the ripening stage. **(A)** Comparison of disease grades among ABLs and the recurrent parents (WYG32 and HG8) against the mixed conidial suspensions of *M. oryzae* isolates. **(B)** The panicle blast disease reactions of ABLs against the mixed conidial suspension 2020-1. ** $P < 0.01$ compared with the recurrent parent by Student's t-test.

Figure 4

Resistance performances of panicle blast for ABLs in a blast nursery. **(A)** Comparison of diseased panicles rates among ABLs and the recurrent parents (WYG32 and HG8). **(B)** The panicle blast disease reactions of partial ABLs in the field in 2019. ** $P < 0.01$ compared with the recurrent parent by Student's t-test.

Figure 5

Breeding high blast-resistant rice variety Yangnonggeng3091 (YNG 3091) with high yield and quality. **(A)** Field performance and milled rice grains of YNG3091. **(B-D)** Comparison of blast resistance level (B), important agronomic traits (C) and grain quality traits (D) between YNG3091 and the control cultivar Huaidao 5 (HD5). * $P < 0.05$, ** $P < 0.01$ compared with HD5 by Student's t-test.

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