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Full Length Article

Marker-assisted Introgression of Broad-spectrum Disease Resistance Genes of *Pigm* and *Xa23* into Rice Restorer

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Abstract

Rice blast and bacterial blight are the two most serious and destructive disease problems in rice production, respectively. In this study, *Pigm* gene with a broad spectrum resistance to blast from GM4 as donor parent were introgressed into eight restorer lines with high quality, leading to the improvement of blast resistance by marker-assisted selection (MAS) and artificial inoculation identification. PCR-based molecular marker M26205 tightly linked to *Pigm* gene was used to select the foreground and the cross populations. A total of 118 rice germplasm accessions from 14 countries were selected to identify, results showed that only eight varieties amplify the fragment with 800bp, whereas others cannot amplify bands, but two bands contained the specific fragment with 1000 bp and 800 bp were amplified in GM4 using the marker M26205. Nine F_7 lines carrying *Pigm* of 49 derivate lines were screened with marker M26205 from eight combinations of GM4 and restorer lines. Nine isolates of *M. oryzae* from Zhejiang were selected to perform the resistance of nine lines to blast. Artificial inoculation demonstrated that nine lines are resistant or moderately resistant to rice blast. The investigation of agronomic traits of nine lines indicated that *Pigm* gene has a negative effect on the number of tiller and 1000 grain weight, but a positive effect on the grain numbers of panicle and the seed setting rate. The line of D3, which possessed the homozygous *Pigm* were crossed with FH450 (as donor parent) carrying *Xa23* gene. The marker C189 tightly linked to *Xa23* gene was used to identify the progeny of the cross generation. 12 stable promising lines possessing high level of resistance to blast and bacterial blight have been identified through marker-assisted breeding.

Keyword: Rice blast; *Pigm* gene; *Xa23* gene; Marker-assisted selection

Introduction

Rice blast and bacterial blight (BB) caused by *Magnaporthe grisea* and *Xanthomonas oryzae pv. oryzae* (*Xoo*) are the two most serious threat causing reduction in rice (*Oryza sativa* L.) production, respectively (Khush and Jena, 2009). Yield loss annually due to rice blast ranged from 10 to 30% of the total yield (Skamnioti and Gurr, 2009). BB is another important disease of rice and the yield reduction of 20% were produced after the rice plants infected by *Xoo* strains at the tillering stage, the yield could be reduced to as much as 50% in the severe year (Mew *et al.*, 1993). In presently, chemical control was effective to control the invasion of *M. oryzae*, but the possible risks could lead to environmental pollution. Therefore, it is the most effective strategy to control diseases through breeding rice varieties conferring the broad-spectrum resistance to the blast and BB.

According to the China Rice Database (http://www.rice data. cn/gene/gene xa. htm), up to June 2012, more than 63 blast resistance locus of 75 major resistance genes have been identified, of which most genes were mapped on chromosomes 6, 11 and 12, respectively. *Pi2*, *Pid2* and *Pi9* have been mapped on chromosome 6 (Yu *et al.,* 1991; Amante-Bordeos *et al.,* 1992; Chen *et al*., 2004), *Pi1*, *Pi7(t)*, *Pi18(t)*, *Pi44(t)*, *Pi34*and *Pik* have been mapped on chromosome 11 (Yu *et al.*, 1991; Wang *et al.,* 1994; Ahn *et al.,* 1996; Chen *et al.,* 1999; Zenbayashi *et al*., 2002; Fjellstrom *et al*., 2004), *Pita*, *Pi6(t)*, *Pi19(t)* and *Pi-41* have been mapped on chromosome 12 (Kiyosawa, 1972; Inukai *et al.*, 1996; Kinoshita, 1997; Yang *et al.*, 2009). Besides *pi21* is a recessive gene, others are dominant. It is well known that molecular mapping of these *R* genes provides tools for MAS in rice breeding for the development of the resistance to blast. For example, two pairs of dominant markers YL155/YL87 and YL183/YL87 of resistant gene *Pita* was established, combined with artificial inoculation to screen *Pita* homozygous genotype breeding lines (Wang *et al.,* 2004). In addition, a set of dominant molecular markers of blast resistance gene *Pib* was developed, including dominant marker Lys145 of the susceptible alleles *Pib* gene and the reported dominant markers. They can rapidly screen the lines containing *Pib* gene from progenies, and effectively identified whether the gene is homozygous in segregating generations (Liu *et al.,* 2008). *Pigm* conferring the broad-

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spectrum resistance to rice blast was identified from a Chinese rice variety, Gumei 4 (GM4), an indica variety from Sichuan. GM4 confer a broader spectrum resistance to blast than other genes including of *Pi1*, *Pi2* and *Pi3*, suggesting it will become a good resistance germplasm in rice resistance breeding. GM4 exhibited high resistance to 29 isolates from different origin in the course of examination of 156 varieties and had the most effective resistance to all isolates (Deng *et al.,* 2006). Therefore, *Pigm* was introduced into restorer line by pedigree and MAS which enables to improve the resistance to blast in the hybrid breeding.

Likewise, BBis the other most destructive disease for rice production. In previous study, some of major resistant genes have been mapped by the linked molecular markers including of *Xa1*, *Xa4*, *Xa5*, *Xa7*, *Xa8*, *Xa13*, *Xa21* and *Xa27* (Sonti, 1998; Rao *et al.,* 2002; Sundaram *et al.,* 2009). Many reports have shown that *Xa21* can be introgressed into rice varieties and hybrid rice parent by MAS for the improvement of the resistance to BB (Pandey *et al.* 2013; Hari *et al.,* 2013). *Xa23*, a single dominant gene which conferred the broad-spectrum resistance to 20 strains, was first identified in *Oryza rufipogon* and mapped on chromosome 11 (Zhang *et al.,* 2001; Zhou *et al.*, 2009).

Throughout educing rice breeding, inherent variability of *M. oryzae* poses a challenge to the utilization of single *R* gene in the rice breeding program, it is one of the trends to pyramid more resistant genes into the same rice variety in rice resistance breeding. Just like genes *Pi1* and *Pi2* conferred broad spectrum blast resistance that were introduced into rice restorer lines Jin23B by marker-assisted selection, gained six lines containing *Pi1*, *Pi2* and itself gene *Pi33*, the resistance evaluation of three genes lines using artificial inoculation was implemented, indicating that the resistant frequency of six lines is 96.74%, higher than the parent which carrying single gene *Pi33* (Chen *et al.,* 2008). Similarly, *Pi9* with the resistance to blast and *Xa23* with the resistance to BB were introduced into the same variety to obtain three lines with double gene (Ni *et al*., 2007).

In this study, GM4 was selected as the donor parent and *Pigm* gene was introgressed into the rice restorer lines through MAS and the pedigree method. Artificial inoculation was performed to evaluate the resistance to blast disease with nine isolates from Zhejiang, China. Additionally, the important agronomic traits were investigated to study the effect of *Pigm* for breeding. Moreover, FH450 which carried *Xa23* were selected as donor parent to cross with the stable lines possessing the homozygous *Pigm* gene in order to develop the elite restorer line with the resistance to blast and BB by pyramiding *Pigm* and *Xa23* genes with the availability of linked markers.

Materials and Methods

Plant Materials and DNA Markers

GM4 possessing *Pigm* gene was selected as donor parent for the resistance to blast. MP3 containing the *Pigm* gene was used as the *japonica* control to detect *Pigm* gene. M61, M233, M38, H404, H506, Xiangwanxian11 (Xwx11), Jiayu99 and Luhui17 with high quality were selected as male parents. The eight combinations between GM4 and restorer lines were constructed in 2004. To confirm the accuracy of MAS, 49 F_7 lines were selected randomly to use in further genotyping and phenotyping experiments. FH450 carrying *Xa23* gene was selected as donor parent for the resistance to BB. All plants were planted at the experimental farm of Zhejiang Academy of Agricultural Sciences, Hangzhou China. A PCR-based Marker R26295 on chromosome 6 from a conserved sequence of *Pigm* gene was selected to identify the *Pigm* in the segregation populations (Deng *et al.,* 2006). An EST marker C189 was selected to identify the *Xa23* gene in the segregation populations (Wang *et al.,* 2005).

Molecular Markers Analysis

Rice genomic DNA was extracted from the plants leaves by the CTAB method (Zhang *et al.,* 1992). The reaction system (20 μL) for PCR analysis consisted of 13.7 μL ddH_2O, 20 ngof template DNA, 2 μL of 10 × PCR buffer, 2.0 μL of 2.5 mMdNTPs, 0.5 μL 10 mM primers and 0.5 U of *Taq* polymerase enzyme (supplied by Shanghai Sangon Biotech Co., Ltd), adding ddH_2O up to 20 μL. Amplification reaction was performed with the following protocol: an initial denaturation at 95°C for 5 min, and then carried out 35 cycles following up 45 s at the denaturation temperature 95°C, 45 s at the annealing temperature 56°C for *Pigm* and 58°C for *Xa23*, and extension at 72 °C for 1 min, and a final cycle 10 min at the extension temperature 72°C. The products were separated by electrophoresis on 3% agarose gel with ethidium bromide in 1% TAE buffer. The gels were taken picture under UV light.

Blast Disease Evaluation

Nine representative blast isolates from Zhejiang province were used in inoculation experiment to determine the plants resistance to blast in this study (Table 2). Two week old seedlings were inoculated with spore suspensions (1×10^5 spores/mL) of the *M. oryzae* isolates. The seedlings inoculated by isolates were placed in a growth chamber for 24 h at 26°C and 90% humidity in darkness and then transferred into to a growth chamber under a12/12 h (day/night) photoperiod at 26°C and 90% humidity for 6 days. To investigate the resistance of the same variety to different isolates, the seedling from the same variety was inoculated with one isolate and the mixture of different isolates by injecting spore suspensions at the seedling stage. The method of inoculation and plants growth were same with the above described. The evaluation of panicle blast severity in plants was performed using a 0–9 scare according to the standard scale ITTP after 7 days.

Evaluation of Agronomic Characteristics

All plants were planted in a paddy field at the experimental farm of Zhejiang Academy of Agricultural Sciences, Hangzhou China. Ten individuals in each plot were taken to investigate the measurement of agronomic traits including of plant height, productive panicles per plant, panicle length, total grains per panicles, filled grain number per panicles, seed setting rate, grain density, 1000 grain weight, grain yield between the selected lines and their parents (Table 3).

Results

Molecular Analysis of GM4, Male Parents and other Rice Germplasm

The marker M26205 was used to detect polymorphisms between GM4 and male parent. Results showed that two bands contained the specific fragment with 1000 bp and 800 bp were amplified in GM4 using M26205, while no bands were amplified in M233, M38, H404, Jiayu99 and Luhui17. Only one band was amplified in M61, with M26205, but there have no *Pigm* gene. M25206 can be used the polymorphic marker to detect the *Pigm* gene between GM4 and other parents (Fig. 1).

To prove the validation of polymorphic marker with M26205 in different rice germplasm, 118 rice accessions from 14 countries, including 97 *indica* varieties, 17 *japonica* varieties and 4 *Javanica* varieties, were selected to perform the polymorphic detection with the marker M26205 (Table 1). GM4 and MP3 possessing *Pigm* gene were used as control in *indica* and *japonica*, respectively. The results showed that two bands contained the specific fragment with 1000 and 800 bp were amplified in GM4 and MP3 using M26205, only one band contained with the specific fragment with 800 bp was amplified in 93072, Ce48, Minxian25, Qianqiannuo, Yesimiao, Yufengzhan2, C135 and Liaodong128. Meanwhile no bands were amplified in other 110 varieties. So, M26205 can be severed as the dominant marker to detect *Pigm* gene effectively.

Molecular Marker Analysis of Different F_7 Lines

49 F_7 lines were selected to detect *Pigm* gene using the marker M26205 from different cross combinations between GM4 and parents. Results showed that two bands contained the specific fragment with 800 and 1000 bp were amplified in nine F_7 lines among them. One F_7 line named as D2 was from the cross GM4/M61, two F_7 lines named as D5 and D6 were from the cross GM4/M233, respectively. And two F_7 lines named as D8 and D9 were from the cross GM4/M38 and GM4/Xiangwanxian11, respectively. Three F7 lines named as D10, D11 and D12 were from the cross GM4/Luhui17, respectively. To identify the homozygous F_7 lines, 20 individual plants from each F_7 lines were randomly

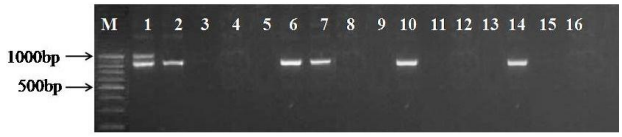

Fig. 1: The polymorphic detection in different rice varieties with the marker M25206M: DNA marker; 1: GM4; 2~16: M61, M233, M38, H404, H506, Xiangwanxian11, Jiayu99, Luhui17, H604, NB, 9311, H706, M11, Taijin8, Xiangwanxian5

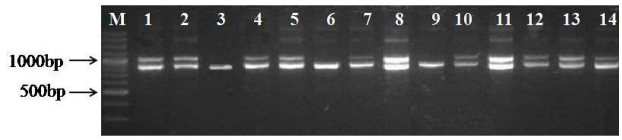

Fig. 2: *Pigm* gene detection in different F_7 lines with the marker M25206M: DNA marker; 1: GM4; 2: MP3, 3-14: D1-D12

selected to detect *Pigm* gene with the marker M26205. Results showed that two bands contained the specific fragments with 800 and 1000 bp were amplified in every individual plant from five F_7 lines including of D3, D5, D8, D11 and D12, which demonstrated that they had homozygous *Pigm* gene, and other four F_7 lines were heterozygous (Fig. 2).

Evaluation of Blast Resistance of the Selected Lines

To evaluate the blast resistance of nine lines carrying *Pigm* gene under artificial inoculation, nine *M. oryzae* isolates collected from Zhejiang Province, China (Table 2). Results showed that GM4 conferred a high level of resistance to all nine strains tested, whereas cultivar H604 was susceptible or moderate susceptible to six *M. oryzae* isolates and only conferred moderate resistance to three isolates. All nine lines exhibited the high resistance to different strains, especially the line of D3 showed a high level of resistance to all isolates. The lines of D5, D11 and D12 were resistant to eight isolates, and only conferred moderate resistant to one isolate. The line of D2 was resistant to five isolates and moderate resistant to four isolates of the nine isolates tested. The line of D6 was resistant to four isolates and moderate resistant to five isolates of the nine isolates tested. The results indicated that *Pigm* gene can provide high and broad-spectrum resistance to *M. oryzae* isolates, different lines carrying *Pigm* gene conferred the different level of resistance to isolates due to the effect of genetic background.

Agronomic Traits of Lines Carrying Homozygous *Pigm* Genotype

In order to assess the breeding effects between GM4 and different male parents, the main agronomic traits of nine F_7

Table 1: Polymorphic detection of *Pigm* gene using M26205 marker for 118 rice germplasm

Rice accessions	Origin	Subspecies	PCR	Rice accessions	Origin	Subspecies	PCR
91499	China	*Indica*	−	Gu154	Cuba	*Indica*	−
93072	China	*Indica*	+	Hongxiang1	China	*Indica*	−
98ning22	China	*Indica*	−	Luhui17	China	*Indica*	−
AmoL3	Iran	*Indica*	−	Hua2-1	China	*Indica*	−
AT85-2	Srilanka	*Indica*	−	Jiayu99	China	*Indica*	−
B4122	Indonesia	*Indica*	−	Mijifu	China	*Indica*	−
BG300	Srilanka	*Indica*	−	Miryang85	Korea	*Indica*	−
BG35-2	Srilanka	*Indica*	−	Minxian25	China	*Indica*	+
BG90-2	Srilanka	*Indica*	−	Nanjing16	China	*Indica*	−
BG94-1	Srilanka	*Indica*	−	Nanjing3736	China	*Indica*	−
CR203	Laos	*Indica*	−	Qianqiannuo	China	*Indica*	+
Gaya byeo	Philippines	*Indica*	−	Shengtai1	China	*Indica*	−
H404	China	*Indica*	−	Shuidaobawang	China	*Indica*	−
H506	China	*Indica*	+	Sixizhang	China	*Indica*	−
H604	China	*Indica*	−	Taohuami	China	*Indica*	−
IR13427-60-1-3-2-2	Philippines	*Indica*	−	Texuanai	China	*Indica*	−
IR1846-300-1	Philippines	*Indica*	−	Texiansimiao1	China	*Indica*	−
IR39334-50-2-1-3-2	Philippines	*Indica*	−	Tiejiaonian	China	*Indica*	−
IR4744-295-2-3	Philippines	*Indica*	−	Xiang85-26	China	*Indica*	−
IR52287-15-2-3-2	Philippines	*Indica*	−	Xiangfuzao32	China	*Indica*	−
IR55419-4	Philippines	*Indica*	−	Xianghui332	China	*Indica*	−
IR5629-64-3	Philippines	*Indica*	−	Xiangwanxian5	China	*Indica*	−
IR57298-174-2-2	Philippines	*Indica*	−	Xiangwanxian11	China	*Indica*	+
IR69727-37-2-1-3-2	Philippines	*Indica*	−	Yandao4	China	*Indica*	−
IR71700-247-1-1-2	Philippines	*Indica*	−	Yangdao6	China	*Indica*	−
IR74	Philippines	*Indica*	−	Yesimiao	China	*Indica*	+
IR8	Philippines	*Indica*	−	Yigengdao	China	*Indica*	−
IR9761-19-1	Philippines	*Indica*	−	Yinchaozhan	China	*Indica*	−
IR9782-111-2-1-2	Philippines	*Indica*	−	Yufengzhan2	China	*Indica*	+
IR9852-93-2-2-2-3	Philippines	*Indica*	−	Yuxian7	China	*Indica*	−
IR-BB54	Philippines	*Indica*	−	Yuxian8	China	*Indica*	−
IR-BB60	Philippines	*Indica*	−	Yuanluzao	China	*Indica*	−
KAU2084	India	*Indica*	−	Yuexinzhan5	China	*Indica*	−
M38	China	*Indica*	−	Zaowanbao24	China	*Indica*	−
M61	China	*Indica*	+	Zaoxuan11	China	*Indica*	−
M233	China	*Indica*	−	Zhaiyeqing	China	*Indica*	−
NDR308	India	*Indica*	−	Zhongerruanzhan	China	*Indica*	−
Pata	Indonesia	*Indica*	−	C135	China	*Japonica*	+
PR106	India	*Indica*	−	C418	China	*Japonica*	−
PSB RC4	Philippines	*Indica*	−	HP121	China	*Japonica*	−
PSB RC28	Philippines	*Indica*	−	Khazar	Iran	*Japonica*	−
PSB RC66	Philippines	*Indica*	−	Jinghui4932	China	*Japonica*	−
R128	China	*Indica*	−	Liaodong128	China	*Japonica*	+
R-18	Unknown	*Indica*	−	Liaojing727	China	*Japonica*	−
R644	Unknown	*Indica*	−	Lunhui01	China	*Japonica*	−
ShweThwe Yin-hyv	Myanmar	*Indica*	−	Lunhui422	China	*Japonica*	−
SW318	Unknown	*Indica*	−	Mengjiaheisi	China	*Japonica*	−
Y134	Malaysia	*Indica*	−	Nange1	China	*Japonica*	−
Baoxiang3	China	*Indica*	−	Taijing8	China	*Japonica*	−
Ce48	China	*Indica*	+	Wuxiangjing14	China	*Japonica*	−
Congai2	China	*Indica*	−	Wuyunjing5	China	*Japonica*	−
Exiang1	China	*Indica*	−	Zhongzuo9567	China	*Japonica*	−
Fengaozhan11	China	*Indica*	−	Nipponbare	Japan	*Japonica*	−
Fenghuazhan	China	*Indica*	−	IAC25	Brazil	*Javanica*	−
Gang16	China	*Indica*	−	IRAT212	France	*Javanica*	−
Gaoyou35	China	*Indica*	−	KOKKO	Brazil	*Javanica*	−
Gengchao1	China	*Indica*	−	M11	Indonesia	*Javanica*	−
GM4	China	*Indica*	++	MP3	China	*Japonica*	++

"+" indicated that one band contained the specific fragment with 800bp was amplified using M26205; "++" indicated that two bands contained the specific fragment with 800bp and 1000bp were amplified; "−" indicated that no bands were amplified

Table 2: Disease evaluation of the nine lines carrying *Pigm* and *GM4* genes to blast isolates

M. oryzae isolates	Cultivars and lines										
	GM4	H604	D2	D3	D5	D6	D8	D9	D10	D11	D12
CH94-10	R	MR	R	R	R	MR	MR	MR	R	R	R
CH2002-067	MR	S	R	R	R	MR	MR	R	R	R	R
CH97-23-2	R	S	R	R	R	R	R	R	R	R	R
CH151	R	MR	R	R	R	R	R	R	R	R	R
CH04-3-1	R	S	R	R	R	R	R	R	R	R	R
CH06-11-1	R	MS	MR	R	MR	MR	MR	R	MR	R	R
CH10-5-1	R	S	MR	R	R	MR	R	MR	MR	R	MR
CH14	R	MR	MR	R	R	MR	R	R	MR	R	R
CH29-2-3	R	MS	MR	R	R	R	R	R	R	MR	R

R: resistant; S: susceptible

lines carrying *Pigm* and the corresponding parents were investigated. The results showed that the mean number of effective tillers and 1000 grain weight of F_7 lines were all less than those of male parents, but the mean number of filled grain and the seed setting rate were more than those of male parents except for the cross of GM4 and M233. These results indicated that GM4 containing *Pigm* gene could be a negative effect on the number of tiller and 1000 grain weight and a positive effect on the number of filled grain and the seed setting rate. However, the grain yield per plant of F_7 lines from the same cross was differed with those parents. For example, D3 (30.13±11.02) was slightly higher than the male parent of M61 (29.38±16.88), but D2 (23.56±3.16) was less than M61. These results indicated that the F_7 lines have a good potential for the development of high yield and resistance to blast after modification by the marker-assisted selection (Table 3).

Pyramiding of *Xa23* and *Pigm* in the Selected Lines

To develop the resistance of rice lines to BB and blast, the line of D3 conferring *Pigm* gene and good agronomic traits were selected as female parent to cross with FH450 containing *Xa23* gene with the broad spectrum resistance to BB. The polymorphic detection showed that M26205 and C189 could be used the dominant marker to detect the *Pigm* and *Xa23* gene in the progeny of the cross D3 and FH450, respectively (Fig. 3). 12 F_3 lines carrying the *Pigm* and *Xa23* gene were obtained by MAS. These results indicated that it is feasible to pyramid *Pigm* and *Xa23* gene into the same genetic ground.

Discussion

Blast is the serious problem in rice production all over the world. MA Shave been successfully used in the development resistance to blast and BB. Breeders can easily introgress resistant genes into the other genetic background with the tightly linked molecular markers. There are many successful instances that breeding has been used for the targeted modification of elite varieties and parental lines of hybrid rice through marker-assisted cross. Balachiranjeevi *et al.* (2013) have introgressed a major dominant gene *Xa21*

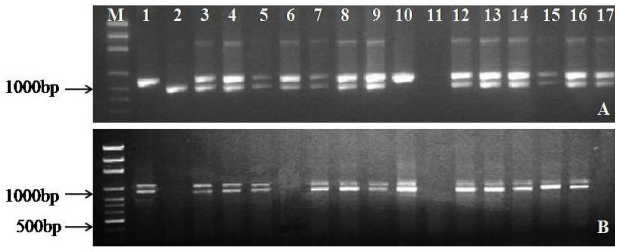

Fig. 3: *Pigm* and *Xa23* gene detection with the marker C189 and M26205. A. *Xa23* gene detection with the marker C189; B. *Pigm* gene detection with the marker M26205. M: DNA marker, 1: D3, 2: FH450, 3-17: F_3 individual plants

with the resistance to BB and *Pi5*4 with the resistance to blast into the maintainer line DDR17B through backcross breeding with MAS. Jiang *et al.* (2015) have been introduced *Pi2* with the broad-spectrum resistance to blast into the elite TGMS line C815S. The field evaluation of the new improved lines and hybrids made from them showed high resistant levels against blast. Singh *et al.* (2012) reported that MAS approach was employed to introduce blast resistance genes Piz-5 and Pi54 into an elite basmati restorer line PRR78. It was reported that blast resistance gene *Pi9* and BB resistance genes *Xa21* and *Xa27* were pyramided in KMDL which is an elite aromatic cultivar by MAS. The improved line confers high resistance to all five *M. oryza* isolates tested and provide resistance or moderate resistance to 25 of 27 *Xoo* strains tested (Luo and Yin, 2013). Near-isogenic lines (NIL)-*Pigm* was constructed with Yangdao6 as recurrent parent and exhibited a good resistance to leaf and panicle blast (Wu *et al.,* 2016). In this study, a PCR-based marker M26205 tightly linked to *Pigm* gene which showed high polymorphism between GM4 and male parents and different rice accessions was used to select the F_7 progeny through MAS and pedigree method. The blast resistance of the improved lines has been developed; especially, the D3 line exhibited a very high level of resistance to nine blast isolates tested in the condition of artificial inoculation and good agronomic traits.

Table 3: Comparison of agronomic traits of improved lines and their parents

Line	Plant height (cm)	Productive panicles per plant	Panicle length (cm)	Total grains per panicles	Filled grain number per panicles	Seed setting rate (%)	Grain density	1000 grain weight (g)	Grain yield per plant (g)
D2	112.17±1.76	9.67±0.58	22.41±2.41	124.33±7.02	105.00±9.64	84.42±0.053	5.60±0.804	23.21±0.28	23.56±3.16
D3	109.83±5.30	10.30±2.08	22.22±2.02	145.67±39.47	127.84±27.50	88.68±0.06	6.56±1.64	22.29±0.74	30.13±11.02
M61	110.59±2.51	10.40±5.41	23.48±1.44	117.63±25.59	103.33±21.04	88.07±0.025	4.92±0.85	27.10±0.03	29.38±16.88
D5	112.17±2.57	10.33±2.52	24.53±1.77	182.67±12.09	132.33±32.50	71.89±0.13	7.45±0.24	23.42±0.71	33.08±15.32
D6	111.17±4.01	8.00±2.00	22.67±2.02	139.00±25.63	111.00±11.53	80.70±0.07	6.23±1.69	23.91±0.08	21.58±7.28
M233	112.82±1.70	11.20±6.02	23.00±1.09	189.52±39.62	125.84±16.14	68.23±0.13	8.28±1.91	20.13±0.023	27.32±12.39
D8	102.67±7.02	10.33±2.08	22.53±2.25	133.67±7.77	101.00±8.54	75.53±0.04	5.96±0.47	29.40±0.62	30.64±6.41
M38	99.78±1.76	12.00±4.64	19.55±0.89	133.32±23.37	77.33±17.94	57.77±0.69	6.87±0.84	31.30±0.026	28.99±12.02
D9	108.50±9.99	8.00±1.00	24.60±4.15	115.67±7.09	90.00±6.56	77.78±0.02	4.77±0.63	26.22±0.24	24.22±2.92
Xwx11	104.02±1.98	9.00±3.00	25.61±2.10	130.96±23.02	78.60±13.58	59.94±0.036	5.11±10.94	27.57±0.03	18.96±5.74
D10	111.97±2.72	8.90±4.44	22.68±0.69	159.04±24.64	109.88±21.77	69.08±0.05	7.01±1.12	26.43±0.02	25.85±9.96
D11	116.83±6.17	9.67±0.58	22.00±1.52	146.33±19.14	113.67±13.87	77.81±0.04	6.63±0.44	26.33±0.42	29.00±4.61
D12	114.00±1.50	8.67±1.53	23.94±2.28	166.67±21.59	129.67±19.40	77.85±0.06	7.04±1.41	25.04±0.20	28.90±8.76
H404	110.61±1.82	9.80±3.56	19.39±1.13	147.96±15.53	92.36±24.36	62.2±0.145	7.60±0.71	28.27±0.026	24.77±8.53

In previous study, the agronomic trait could be affected after the introgression of resistance gene into the recurrent parents. For example, the plant height of NIL-*Pi2* was shorter by 5–9 cm than that of the recurrent parent, the day of 50% flowering of NIL-*Pi2* less than it, total spikelets per plant of NIL-Pi2 was significantly lower than it, indicating the alleles of undesirable agronomic traits were closely linked to *Pi2* gene (Wu *et al.,* 2016). T5105, an improved line, produced higher yield than the recurrent parent of KMDL105 and retain similar good grain quality to KMDL105 (Luo and Yin, 2013). In this study, the mean number of effective tillers and 1000 grain weight of the improved line were all less than those of male parents, but the mean number of filled grain and the seed setting rate were more than those of male parents, indicating that the undesirable trait of segment were also introduced into the progeny of F_7 lines.

Conclusion

BB is the other serious disease in rice production. *Xa23* has been identified from a wild rice (*O. rufipogon*) confers broad-spectrum resistance against BB and widely adopted in rice breeding programs (Zhang *et al.,* 2001; Zhou *et al.,* 2009). Pyramiding of different resistance gene against blast and BB was the effective way to improve the resistance of rice variety. In this study, an improved line D3 conferring *Pigm* gene and good agronomic traits were selected to cross with FH450 containing *Xa23* gene. PCR-based marker C189 linked with *Xa23* gene and M26205 linked with *Pigm* gene were performed to detect in the progeny of F_3 generation. The results indicated that *Xa23* and *Pigm* can be pyramided in the same genetic background through MAS.

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