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Introgression of bacterial blight (BB) resistance genes *Xa7* and *Xa21* into popular restorer line and their hybrids by molecular marker-assisted backcross (MABC) selection scheme

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Yihui1577 is an elite restorer line widely used in hybrid rice production in China, however, both the restorer and their derived hybrids are susceptible to bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). In order to overcome this problem, we had introgressed two resistant genes *Xa7* and *Xa21* into Yihui1577 by marker-assisted backcross (MABC) with foreground selection scheme to speed up the process. Six breeding lines with different BB resistance genes: HH1202 (*Xa7*), HH1203 (*Xa7*), HH1204 (*Xa21*), HH1205 (*Xa21*), HH1206 (*Xa7+Xa21*) and HH1207 (*Xa7+Xa21*) were selected and crossed with four CMS and one TGMS lines. Seven most virulent and prevalent *Xoo* strains (PXO61, PXO99, ZHE173, GD1358, FuJ, YN24 and HeN11) from the Philippines and different provinces of China were inoculated for evaluating the BB-resistance of the selected lines and their derived hybrids. The results reveal that the two lines and their derived hybrids with single resistance gene *Xa7* were resistant against six of the seven *Xoo* strains, except for PXO99. The lines with single resistance gene *Xa21* were only susceptible to the *Xoo* strain FuJ, but some of their derived hybrids were susceptible to the *Xoo* strains FuJ and GD1358. Interestingly, the pyramiding lines carrying the two resistance genes *Xa7* and *Xa21* and also their derived hybrids were resistant against all the seven *Xoo* strains. The data of agronomic and grain quality characteristics demonstrated that the selected lines were similar to that of the recurrent parent Yihui1577. Corrective measures taken by way of introgression of BB-resistance genes: *Xa7* and *Xa21* into the popular restorer line, Yihui1577 through MABC approach for enhancing the BB-resistance level was effective and timely.

Key words: Bacterial blight, resistance gene, *Xa7* and *Xa21*, MABC, inoculation and reaction, agronomic traits, grain quality.

INTRODUCTION

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*) is one of the most destructive diseases widely prevalent in rice-growing regions of China, causing significant reduction in rice production and grain quality

losses in rice hybrids. BB is considered to be the second largest disease after blast covering all parts of China excluding Xinjiang province (Zhang et al., 2002). Breeding for disease resistance is the most effective and economical method for control of BB creating a neutral impact on the environment (Khush et al., 1989). Prior to 1980s in China, BB was effectively controlled by deploying the *Xa3* and *Xa4* resistance genes in rice varieties. However, the breakdown of resistance of these

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genes to new virulent BB strains had created BB outbreaks in rice growing areas in China (Xu et al., 2004).

Until 2010, 38 BB-resistant genes have been reported and registered, and as many as five dominant resistant genes: *Xa1*, *Xa21*, *Xa23*, *Xa3/Xa26*, *Xa27* and two recessive resistant genes (*xa5* and *xa13*) have been cloned (Yoshimura et al., 1998; Song et al., 1995; Wang et al., 2009; Sun et al., 2004; Xiang et al., 2006; Gu et al., 2004; Blair et al., 2003; Chu et al., 2006). Amongst them, two dominant genes: *Xa21* and *Xa7* provide durable resistance to *Xoo* races. Broad spectrum resistance gene *Xa21* was identified from the wild rice (*Oryza longistaminata*) that was mapped on chromosome 11 and also it happens to be the first cloned BB resistance gene (Song et al., 1995). Likewise, *Xa7* is another broad spectrum resistance gene that was originally identified in rice cultivar DV85 (Sidhu et al., 1978) and was later on fine mapped (Chen et al., 2008).

Yihui1577 is one of the elite restorer lines used in hybrid rice program of China. As many as seven hybrids in which Yihui1577 has been used as male parent were certified and released in South China, especially on account of its high yield and good grain quality. Unfortunately, all these hybrids were found to be susceptible to BB because both Yihui1577 and the male sterile lines that were used as female parents in hybrid rice breeding program were not resistant against the pathogen. Therefore, one of the key objectives of this study was to introgress *Xa7* and *Xa21* into the Yihui1577 background by marker assisted backcross (MABC) with foreground selection scheme. And develop the new BB resistant hybrids to replace the existing susceptible hybrids without compromising the grain yield and quality.

MATERIALS AND METHODS

Six BB-resistance lines previously developed at National Center of Plant Gene Research, Huazhong Agricultural University, Wuhan, China were used in this study as donors that include two lines each carrying single resistance gene *Xa7*, *Xa21* and both *Xa7* + *Xa21* together. A total of 30 hybrids were produced by crossing these six BB resistant lines with five male sterile lines. Seven most prevalent and virulent strains of *Xoo* were inoculated artificially in field for evaluating the BB-resistance levels of the selected restorer lines and their derived hybrids.

Huahui20 carrying *Xa7* and *Xa21*, was used as a donor for BB resistance genes to be transferred to popular restorer line: Yihui1577 through MABC breeding approach. Recipient, Yihui1577 is one of the commercial restorer lines in South China with high combining ability, but is susceptible to BB. Donor, Huahui20 is a breeding line derived from pyramiding crosses among Minghui63, IRBB7 (*Xa7*) and IRBB21 (*Xa21*).

Transfer of the *Xa7* and *Xa21* genes by molecular marker-assisted backcross (MABC) with foreground selection scheme into Yihui1577

Yihui1577 was used as the female parent and was crossed with

Huahui20 in 2005 to obtain F₁ plants. True F₁ plants were used as the pollen plants and then backcrossed to Yihui1577 to obtain BC₁F₁ seeds. Genotype of each BC₁F₁ plant was determined by using tightly linked SSR/STS molecular markers and marker-assisted selection (MAS) was carried out. SSR marker, RM20582 was used in the presence of *Xa7* to map a 0.14-cM interval between the markers RM20582 and RM20593 on chromosome 6 (Chen et al., 2008). STS marker, pTA248 was used in the presence of *Xa21* (Huang et al., 1997). The BC₁F₁ plants with a genotype of *Xa7xa7/Xa21xa21* were further backcrossed to Yihui1577 to produce the BC₂F₁ population. Consecutive backcrossing and MAS was employed in each generation. At BC₃F₁ generation, plants were selfed to produce BC₃F₂ population. Homozygous plants carrying *Xa7*, *Xa21* and both of them were selected in BC₃F₂ to produce BC₃F₃ families. 20 plants of each breeding BC₃F₃ line were transplanted with a spacing of 16.7 by 20 cm between plants and rows. PCR analyses were done again to confirm the homozygous target genes. Two lines of each genotype with a phenotypically similar recurrent parent (Yihui1577) were selected and consecutively selfed to BC₃F₆. Finally, six selected breeding lines, HH1202 and HH1203 with *Xa7Xa7* genotype, HH1204 and HH1205 (*Xa21Xa21*), HH1206 and HH1207 (*Xa7Xa7/Xa21Xa21*) were used for cross testing, resistance and agronomic trait evaluations.

Resistance evaluation through artificial BB inoculation

Six breeding lines, recurrent parent (Yihui1577), donor parent (Huahui20), control varieties (IRBB7, IRBB21, and IR24), five male sterile lines (C815S, Jinke1A, Chuannong1A, Jufeng A and Chuan23A) and their F_{1s} (Tables 2 to 4), were grown in the field of Experimental Farm of Huazhong Agricultural University (HZAU), Wuhan, China during the summer seasons of 2009 and 2010. Germinated seeds were sown in seed beds in early May. 25 days old seedlings was uprooted and transplanted as single plant per hill in the main field. Seven separate experiments were arranged for evaluation with seven *Xoo* strains. Each line comprised of eight plants in one row planted with a spacing of 16.7 × 26.7 cm. Amongst the seven *Xoo* strains, ZHE173 and GD1358 were the most prevalent and virulent in indica rice growing area of Southern China, while PXO61 and PXO99 were from Philippines. The remaining three new *Xoo* strains were YN24, FuJ and HEN11 from Yunnan, Fujian and Henan Provinces of Southwest China (Liu et al., 2007), respectively. All the seven *Xoo* strains that were provided by Department of Plant Pathology, Nanjing Agricultural University, were prepared following the method described by Maruthasalam et al. (2007). Plants were inoculated with the bacterial suspension at a density of 10⁹ cells/ml at maximum tillering stage of plant development. All eight hills with three leaves per hill for each line were inoculated following the procedure described by Jennings et al. (1979). The lesion length was carefully measured in cm by scale after three weeks of inoculation on all inoculated leaves. Plant reaction to bacteria was scored according to Table 1, which is a standard method for scoring rice variety (including inbred and hybrid) reaction to BB in China National Rice Trial Program.

PCR amplification system

Each 20 µl PCR reaction mixture contained 20 ng genomic DNA, 10 mM Tris-HCl pH 9.0, 50 mM KCl, 2.5 mM MgCl₂, 2 mM dNTPs, 10 µM each of the primer pair and 1.5 units Taq DNA polymerase. PCR reaction were performed in a Thermocycler using the following file: An initial denaturation was performed at 94°C for 5 min prior to 35 cycles of denaturation at 94°C (1 min), annealing at 55°C (1 min) for RM20582 or 60°C (1 min) for pTA248, and extension at 72°C (1 min)

Table 1. Disease reaction scores for BB resistance.

Score	Lesion length (cm)	Resistance level
0	<1.0	Highly resistance (HR)
1	1.1 to 3.0	Resistance (R)
3	3.1 to 5.0	Moderate resistance (MR)
5	5.1 to 12.0	Moderate susceptible (MS)
7	12.1 to 20.0	Susceptible (S)
9	>20.0	Highly susceptible (HS)

followed by a final extension of 10 min at 72°C. Polymorphism in the PCR products was detected after electrophoresis on 2.5% agarose gel for STS marker (pTA248) or 4% polyacrylamide gel for SSR marker RM20582 in 1×Tris/Borate/EDTA (TBE) buffer.

Evaluation of agronomic and grain quality traits

Six selected lines and Yihui1577 were grown in a randomized block design with three replicates in the Experimental Farm of HZAU, Wuhan, China during summer season of 2009. 30 plants of each entry were planted over three rows with a plant to row spacing of 16 by 20 cm. All standard agronomic management and plant protection practices were adopted to ensure a good crop growth. Data on agronomic traits like heading date, plant height, productive panicles, average panicle length, spikelets per plant, spikelet fertility percent and 1000-grain weight were recorded as per the standard evaluation system (SES) (IRRI, 1996).

Grain quality traits were measured after harvest at 12 to 14% moisture content. Grain quality characters that were analyzed were brown rice recovery rate (%), milled rice recovery rate (%), head rice recovery rate (%), milled head rice length (mm), length and breadth ratio, chalkiness degree (%), gel consistency (mm), alkali spreading (score) and amylose content (%). The grain samples were dehulled to brown rice using a Satake Rice Machine (Satake Corp. Japan) and then ground to pass through a 100-mesh sieve on a Cyclone Sample Mill (UDY Corp., Fort Collins, CO), and milled rice recovery rate, head rice recovery rate, length and breadth ratio, chalkiness degree were measured with an automatic machine (JMW12, China). Gel consistency was determined by the method of Cagampang et al. (1973). Alkali spreading was assayed following the procedure of Bhattacharya (1979). Amylose content (AC) was measured using the method of Tan et al. (1999). A standard curve made using rice samples of known amylose content was used to estimate the AC of each sample and for each sample, the measurements were taken in triplicate and their averages were taken.

RESULTS

Molecular marker assisted selection of *Xa7* and *Xa21* resistance genes

Analysis of polymorphism of *Xa7* and *Xa21* resistance gene between Yihui1577 and Huahui20 by RM20582 and pTA248 showed two markers to be co-dominant and polymorphic. We selected six lines with different resistance genes and designated them as HH1202 (*Xa7*),

HH1203 (*Xa7*), HH1204 (*Xa21*), HH1205 (*Xa21*), HH1206 (*Xa7+Xa21*) and HH1207 (*Xa7+ Xa21*) based on marker data, BB resistance screening and phenotypic evaluation. Molecular identification and detection of representative line, HH1206 carrying *Xa7* and *Xa21* genes is depicted in Figure 1.

Disease reaction of selected lines and controls to seven *Xoo* strains

11 entries comprising Yihui1577 (recipient parent), six selected lines (HH1202, HH1203, HH1204, HH1205, HH1206 and HH1207), Huahui20 (donor parent) and three controls (IRBB7, IRBB21 and IR24) were evaluated for their resistance to seven strains of *Xoo*, PXO61 and PXO99 from the Philippines, ZHE173, GD1358, YN24, FuJ and HEN11 from China during summer seasons of 2009 and 2010 in the Experimental Farm Fields of Huazhong Agricultural University, Wuhan, China (Table 2). Results show that IR24 (susceptible control) was highly susceptible to all seven strains with the average lesion length of >12 cm, and it also indicated that inoculated pathogen reaction was stable and the pathogenicity of seven stains was normal. Resistance gene *Xa7* (IRBB7) was effective against PXO61, ZHE173, GD1358, YN24 and FuJ, but was not resistant against PXO99 and HEN11. While resistance gene *Xa21* (IRBB21) was resistant to six strains, except FuJ. Recurrent parent, Yihui1577 was classified as moderate susceptible, susceptible or highly susceptible towards *Xoo* strains PXO61, PXO99, ZHE173, GD1358, FuJ and YN24 with an average lesion length ranging from 7.20 to 23.89 cm over the two years of screening. Interestingly, Yihui1577 was resistant against HEN11 strain. Selected lines, HH1202 and HH1203 carrying single resistance gene *Xa7* were moderately resistant or resistant or highly resistant to six *Xoo* stains with an average lesion length of 0.29 to 3.35 cm over two years, but was not resistant to PXO99. HH1204 and HH1205 carrying single resistance gene *Xa21* were moderately resistant or resistant or highly resistant to six *Xoo* stains with the average lesion length of 0.13 to 4.82 cm over two years, but was susceptible to FuJ. The pyramiding lines, HH1206 and HH1207 carrying two R genes together (*Xa7* and *Xa21*) were moderately resistant, resistant or highly resistant to all seven *Xoo* stains in this study over two years with an average lesion length in the range of 0.11 to 3.50 cm. These results indicate that the resistance effect of two resistance gene pyramiding lines were much superior to the lines carrying single resistance gene.

BB resistance reaction in F_1 rice hybrids

Two cytoplasm male sterile (CMS) lines, Jinke 1A and

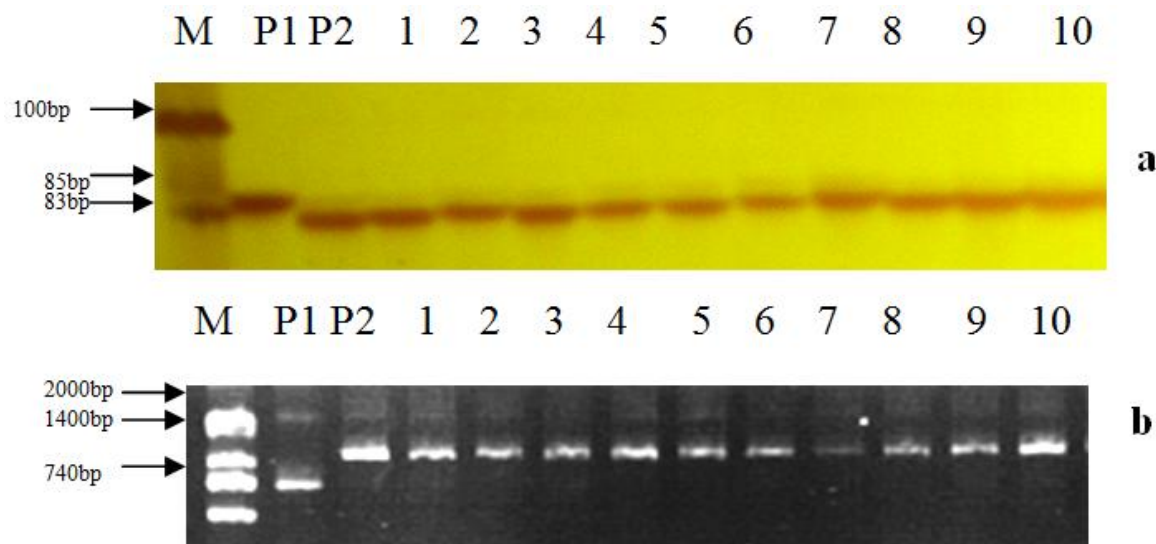


Figure 1. PCR detection of *Xa7* (a) and *Xa21* (b) in selected BB resistant line, HH1206. M, DNA marker; P1, recipient; P2, donor parent; lanes 1 to 10, 10 Plants of HH1206.

Chuannong1A, and one thermo-sensitive genic male sterile (TGMS) line, C815S, were used as female parents crossed with seven male parents that comprises six selected BB-resistant line (HH1202, HH1203, HH1204, HH1205, HH1206 and HH1207) and Yihui1577 (check) in spring season of 2009. A total of 21 F_1 hybrids were evaluated for BB resistance in field along with the three female parents through artificial inoculation with seven *Xoo* strains in summer season of 2009 (Table 3). Results reveal that three male sterile lines were found to be susceptible to all seven *Xoo* strains. The lesion length of Chuannong1A was longest, followed closely by Jinke1A and that of C815S was relatively shorter. The hybrids in which the original recipient parent, Yihui1577, was used as male parent were susceptible to all seven *Xoo* strains. The levels of BB-resistance for hybrids in which the selected BB-resistant lines were used as male parents were significantly increased in comparison with the hybrids of Yihui1577 (original recipient parent) used as male parent. Among the crosses in which C815S was used as female parents, the hybrids with single R gene *Xa7* were resistant against six of the seven *Xoo* strains, except PXO99, whereas the hybrids with single R gene *Xa21* were resistant to six of the seven *Xoo* strains, except FuJ and the hybrids with two R genes *Xa7* and *Xa21* were resistant against all seven *Xoo* strains. When Chuannong1A which showed lower level of BB-resistance was used as a female parent, the resistance spectrum became narrow. Hybrids with single R gene *Xa7* or *Xa21* were resistant against four of the seven *Xoo* strains (Table 3), whereas the hybrids with two R genes *Xa7* and *Xa21* were resistant to six of the seven *Xoo* strains, except FuJ.

Another two CMS lines, Jufeng A and Chuan23A were crossed with six selected BB-resistant lines and Yihui1577 in spring season of 2010, and their 14 hybrids were evaluated for BB resistance during summer season of 2010 in a similar manner as mentioned above. Results indicate that two CMS were found to be susceptible to all seven *Xoo* strains (Table 4). As compared to the results among BB-resistance of hybrids in 2009, some differences had appeared in the resistance spectrum. All 12 hybrids with single or two R genes in which the selected BB-resistant lines were used as male parent were not resistant against both PXO99 and YN24. However, the hybrids with single R gene, *Xa21* were highly susceptible to the *Xoo* strain, FuJ.

In all the 30 hybrids, six selected BB-resistant lines that were evaluated for BB resistance in the study were found to be resistant against the two *Xoo* strains, ZHE173 and GD1358 that are widely prevalent in hybrid rice production area of Southern China.

Phenotypic and grain quality evaluation

Agronomic evaluation trial of the selected BB resistant lines was conducted at the Experimental Farm of HZAU, Wuhan in 2009 and the results are given in Table 5. Most agronomic traits showed non-significant difference between the selected lines and the recurrent parent Yihui1577 for all eight traits tested. However, some of the selections had shown significant difference with Yihui1577. As compared to Yihui1577, HH1202 had significant increase in the yield per plant, HH1205 had significant increase in the effective panicles per plant, and HH1206

Table 2. Disease reaction (average lesion length in cm \pm S.E.) and resistance levels of selected BB resistant lines, recurrent parent and control varieties upon inoculation with seven *Xoo* strains over two consecutive years (2009 to 2010) at Wuhan.

Year	Line	Generation	Genotype of R gene	PX061	PX099	ZHE173	GD1358	YN24	FuJ	HEN11
2009	Yihui1577		Unknown	4.07 \pm 1.23 (MR)	12.76 \pm 2.31 (S)	4.05 \pm 1.94 (MR)	23.89 \pm 3.68 (HS)	14.59 \pm 2.02 (S)	16.34 \pm 2.98 (S)	2.38 \pm 1.08 (R)
	HH1202	BC ₃ F ₆	<i>Xa7Xa7</i>	0.47 \pm 0.33 (HR)	15.07 \pm 2.83 (S)	0.24 \pm 0.11 (HR)	0.69 \pm 0.28 (HR)	0.75 \pm 0.28 (HR)	3.31 \pm 1.64 (MR)	2.77 \pm 0.55 (R)
	HH1203	BC ₃ F ₆	<i>Xa7Xa7</i>	0.43 \pm 0.28 (HR)	11.84 \pm 2.70 (S)	0.44 \pm 0.21 (HR)	0.29 \pm 0.18 (HR)	1.03 \pm 0.67 (R)	3.04 \pm 1.08 (MR)	1.55 \pm 0.72 (R)
	HH1204	BC ₃ F ₆	<i>Xa21Xa21</i>	0.21 \pm 0.18 (HR)	2.19 \pm 1.50 (R)	0.23 \pm 0.15 (HR)	0.91 \pm 1.73 (HR)	0.88 \pm 0.50 (HR)	13.88 \pm 3.63 (S)	0.58 \pm 0.17 (HR)
	HH1205	BC ₃ F ₆	<i>Xa21Xa21</i>	0.22 \pm 0.10 (HR)	2.43 \pm 1.69 (R)	0.16 \pm 0.10 (HR)	0.44 \pm 0.28 (HR)	0.69 \pm 0.38 (HR)	12.24 \pm 1.12 (S)	0.48 \pm 0.21 (HR)
	HH1206	BC ₃ F ₆	<i>Xa7Xa7/Xa21Xa21</i>	0.18 \pm 0.08 (HR)	1.28 \pm 0.61 (R)	0.13 \pm 0.05 (HR)	0.29 \pm 0.13 (HR)	0.36 \pm 0.21 (HR)	1.69 \pm 0.75 (R)	0.52 \pm 0.18 (HR)
	HH1207	BC ₃ F ₆	<i>Xa7Xa7/Xa21Xa21</i>	0.16 \pm 0.08 (HR)	1.82 \pm 1.10 (R)	0.16 \pm 0.07 (HR)	0.36 \pm 0.22 (HR)	0.30 \pm 0.14 (HR)	1.88 \pm 0.69 (R)	0.61 \pm 0.58 (HR)
	Huahui20		<i>Xa7Xa7/Xa21Xa21</i>	0.32 \pm 0.16 (HR)	4.49 \pm 1.90 (MR)	0.38 \pm 0.22 (HR)	2.89 \pm 2.03 (R)	1.71 \pm 1.01 (R)	3.12 \pm 1.29 (MR)	1.45 \pm 0.46 (R)
	IRBB7		<i>Xa7Xa7</i>	0.35 \pm 0.31 (HR)	12.37 \pm 2.28 (S)	0.74 \pm 1.16 (HR)	1.29 \pm 0.73 (R)	1.72 \pm 0.94 (R)	0.42 \pm 0.60 (HR)	6.72 \pm 2.05 (MS)
	IRBB21		<i>Xa21Xa21</i>	1.77 \pm 1.03 (R)	1.94 \pm 0.79 (R)	1.59 \pm 0.65 (R)	3.94 \pm 1.51 (MR)	1.25 \pm 0.76 (R)	17.86 \pm 2.88 (S)	1.12 \pm 0.32 (R)
IR24		Unknown	15.45 \pm 3.97 (S)	15.62 \pm 3.83 (S)	21.53 \pm 3.27 (HS)	20.88 \pm 3.07 (HS)	--	21.26 \pm 3.86 (HS)	16.39 \pm 3.13 (S)	
2010	Yihui1577		Unknown	7.20 \pm 2.49 (MS)	13.13 \pm 3.70 (S)	7.90 \pm 2.59 (MS)	9.45 \pm 1.75 (MS)	10.15 \pm 2.31 (MS)	21.17 \pm 3.43 (HS)	2.84 \pm 0.72 (R)
	HH1202	BC ₃ F ₈	<i>Xa7Xa7</i>	1.54 \pm 0.82 (R)	13.59 \pm 2.98 (S)	0.36 \pm 0.43 (HR)	1.59 \pm 0.38 (R)	3.35 \pm 1.31 (MR)	2.52 \pm 1.02 (R)	2.68 \pm 0.39 (R)
	HH1203	BC ₃ F ₈	<i>Xa7Xa7</i>	0.79 \pm 0.69 (HR)	16.29 \pm 4.20 (S)	0.93 \pm 0.81 (HR)	0.58 \pm 0.18 (HR)	2.29 \pm 0.80 (R)	2.60 \pm 0.93 (R)	1.71 \pm 0.43 (R)
	HH1204	BC ₃ F ₈	<i>Xa21Xa21</i>	0.28 \pm 0.42 (HR)	4.82 \pm 1.62 (MR)	0.13 \pm 0.07 (HR)	0.90 \pm 0.51 (HR)	1.73 \pm 0.55 (R)	13.88 \pm 3.63 (S)	0.74 \pm 0.23 (HR)
	HH1205	BC ₃ F ₈	<i>Xa21Xa21</i>	0.29 \pm 0.40 (HR)	2.79 \pm 0.59 (R)	0.17 \pm 0.16 (HR)	0.42 \pm 0.26 (HR)	1.47 \pm 0.66 (R)	12.24 \pm 1.12 (S)	0.60 \pm 0.24 (HR)
	HH1206	BC ₃ F ₈	<i>Xa7Xa7/Xa21Xa21</i>	0.15 \pm 0.16 (HR)	3.09 \pm 1.68 (MR)	0.11 \pm 0.04 (HR)	0.21 \pm 0.20 (HR)	0.86 \pm 0.42 (HR)	1.57 \pm 0.78 (R)	0.78 \pm 0.37 (HR)
	HH1207	BC ₃ F ₈	<i>Xa7Xa7/Xa21Xa21</i>	0.17 \pm 0.13 (HR)	3.50 \pm 1.08 (MR)	0.12 \pm 0.07 (HR)	0.23 \pm 0.24 (HR)	0.98 \pm 0.44 (HR)	1.59 \pm 0.76 (R)	0.87 \pm 0.43 (HR)
	Huahui20		<i>Xa7Xa7/Xa21Xa21</i>	0.76 \pm 0.56 (HR)	7.81 \pm 2.27 (MS)	0.95 \pm 0.60 (HR)	0.62 \pm 0.22 (HR)	7.68 \pm 2.16 (MS)	1.87 \pm 0.61 (R)	0.56 \pm 0.18 (HR)
	IRBB7		<i>Xa7Xa7</i>	0.45 \pm 0.44 (HR)	9.96 \pm 3.98 (MS)	0.28 \pm 0.28 (HR)	0.62 \pm 0.58 (HR)	7.61 \pm 2.56 (MS)	0.10 \pm 0.00 (HR)	13.87 \pm 2.01 (S)
	IRBB21		<i>Xa21Xa21</i>	1.43 \pm 0.41 (R)	5.00 \pm 1.92 (MR)	2.64 \pm 1.12 (R)	8.27 \pm 1.91 (MS)	6.77 \pm 2.38 (MS)	20.63 \pm 3.41 (HS)	2.62 \pm 0.75 (R)
IR24		unknown	15.12 \pm 3.41 (S)	17.13 \pm 2.28 (S)	24.39 \pm 2.57 (HS)	14.58 \pm 3.85 (S)	18.32 \pm 3.32 (S)	24.54 \pm 4.22 (HS)	12.65 \pm 1.22 (S)	

R, Resistant; MR, mediate resistant; HR, highly resistant; S, susceptible; MS, mediate susceptible; HS, highly susceptible.

had significant increase in the grains per plant and yield per plant, respectively, whereas HH1204 had significant decrease in the seed setting.

Eight grain quality characteristics of six selected lines showed non-significant differences as compared to the recurrent parent, Yihui1577 (Table 5). These results indicate that six selected BB-resistant lines had recovered largely the

recipient parental genetic background for grain quality traits studied.

DISCUSSION

Currently, we understand that the BB resistance genes often breakdown if proper deployment

strategy of BB resistance genes is not done. We know the application of a single resistance gene could control rice BB but the pathogen appears to be fast evolving to overcome the resistance gene with more virulent new pathogenic races. *Xa4* and *Xa3* were two popular BB-resistance genes in *indica* hybrid rice in China before the 1980s since the *indica* restorer lines were introduced from IRR1

Table 3. Disease reaction (average lesion length in cm \pm S.E.) and resistance levels of hybrids and male sterile lines upon inoculation with seven *Xoo* strains in 2009 at Wuhan.

Entry	Genotype of R gene	PXO61	PXO99	ZHE173	GD1358	YN24	FuJ	HEN11
C815S	Unknown	10.22 \pm 1.01 (MS)	15.24 \pm 1.13 (S)	16.21 \pm 1.23 (S)	30.62 \pm 1.78 (HS)	18.92 \pm 1.20 (S)	25.96 \pm 2.32 (HS)	13.20 \pm 0.68 (S)
C815S/Yihui1577	Unknown	4.57 \pm 1.90 (MR)	9.85 \pm 2.37 (MS)	5.16 \pm 1.49 (MS)	23.29 \pm 3.20 (HS)	11.54 \pm 3.34 (MS)	17.74 \pm 3.21 (S)	5.60 \pm 2.03 (MS)
C815S/HH1202	<i>xa7Xa7</i>	1.17 \pm 0.87 (R)	14.17 \pm 2.98 (S)	0.51 \pm 0.43 (HR)	1.53 \pm 0.86 (R)	1.45 \pm 0.62 (R)	4.93 \pm 1.71 (MR)	3.15 \pm 1.49 (MR)
C815S/HH1203	<i>xa7Xa7</i>	0.39 \pm 0.27 (HR)	14.20 \pm 2.87 (S)	0.79 \pm 0.58 (HR)	1.14 \pm 0.34 (R)	0.66 \pm 0.24 (HR)	4.89 \pm 2.26 (MR)	2.54 \pm 1.13 (R)
C815S/HH1204	<i>xa21Xa21</i>	0.57 \pm 0.33 (HR)	1.93 \pm 1.09 (R)	0.75 \pm 0.48 (HR)	2.87 \pm 1.92 (R)	0.65 \pm 0.25 (HR)	15.69 \pm 2.65 (S)	1.73 \pm 0.97 (R)
C815S/HH1205	<i>xa21Xa21</i>	0.30 \pm 0.17 (HR)	2.79 \pm 0.70 (R)	0.20 \pm 0.08 (HR)	0.87 \pm 0.32 (HR)	0.75 \pm 0.32 (HR)	16.37 \pm 4.08 (S)	0.82 \pm 0.32 (HR)
C815S/HH1206	<i>xa7Xa7/xa21Xa21</i>	0.17 \pm 0.09 (HR)	2.57 \pm 0.79 (R)	0.17 \pm 0.07 (HR)	0.68 \pm 0.33 (HR)	0.97 \pm 0.36 (HR)	3.16 \pm 1.29 (MR)	0.85 \pm 0.27 (HR)
C815S/HH1207	<i>xa7Xa7/xa21Xa21</i>	0.18 \pm 0.08 (HR)	4.23 \pm 2.20 (MR)	0.19 \pm 0.09 (HR)	0.98 \pm 0.99 (HR)	0.43 \pm 0.17 (HR)	4.37 \pm 2.01 (MR)	1.62 \pm 0.77 (R)
Jinke1A	Unknown	15.96 \pm 1.25 (S)	20.67 \pm 2.08 (S)	13.01 \pm 1.54 (S)	35.56 \pm 2.33 (HS)	24.68 \pm 1.86 (HS)	30.68 \pm 2.79 (HS)	18.75 \pm 1.09 (S)
Jinke1A/Yihui1577	Unknown	4.58 \pm 1.31 (MR)	13.94 \pm 2.63 (S)	5.70 \pm 2.75 (MS)	28.07 \pm 3.71 (HS)	14.79 \pm 3.21 (S)	20.07 \pm 2.81 (S)	7.17 \pm 1.53 (MS)
Jinke1A/HH1202	<i>xa7Xa7</i>	2.00 \pm 0.99 (R)	15.29 \pm 3.07 (S)	0.51 \pm 0.41 (HR)	3.74 \pm 1.35 (MR)	0.45 \pm 0.27 (HR)	4.88 \pm 2.00 (MR)	4.69 \pm 1.35 (MR)
Jinke1A/HH1203	<i>xa7Xa7</i>	0.77 \pm 0.50 (HR)	14.72 \pm 3.08 (S)	0.54 \pm 0.23 (HR)	1.96 \pm 0.61 (R)	0.25 \pm 0.10 (HR)	5.03 \pm 1.71 (MR)	4.41 \pm 0.94 (MR)
Jinke1A/HH1204	<i>xa21Xa21</i>	0.54 \pm 0.38 (HR)	3.59 \pm 1.66 (MR)	0.77 \pm 1.54 (HR)	13.14 \pm 5.40 (S)	1.16 \pm 0.80 (R)	10.62 \pm 6.11 (MS)	1.66 \pm 0.58 (R)
Jinke1A/HH1205	<i>xa21Xa21</i>	0.28 \pm 0.16 (HR)	2.91 \pm 1.79 (R)	0.15 \pm 0.06 (HR)	13.25 \pm 3.79 (S)	1.69 \pm 1.31 (R)	6.29 \pm 1.92 (MS)	1.32 \pm 0.53 (R)
Jinke1A/HH1206	<i>xa7Xa7/xa21Xa21</i>	0.31 \pm 0.21 (HR)	2.68 \pm 1.31 (R)	0.13 \pm 0.06 (HR)	1.37 \pm 0.83 (R)	0.46 \pm 0.22 (HR)	4.50 \pm 1.83 (MR)	1.73 \pm 0.51 (R)
Jinke1A/HH1207	<i>xa7Xa7/xa21Xa21</i>	0.27 \pm 0.11 (HR)	2.29 \pm 0.55 (R)	0.15 \pm 0.06 (HR)	1.38 \pm 0.55 (R)	0.43 \pm 0.24 (HR)	4.94 \pm 1.33 (MR)	1.84 \pm 0.77 (R)
Chuannong1A	Unknown	30.58 \pm 2.53 (HS)	35.22 \pm 2.89 (HS)	36.65 \pm 2.33 (HS)	36.17 \pm 2.18 (HS)	35.20 \pm 2.26 (HS)	35.30 \pm 3.75 (HS)	25.44 \pm 3.12 (HS)
Chuannong1A/Yihui1577	Unknown	12.91 \pm 2.93 (S)	18.62 \pm 4.24 (S)	15.59 \pm 3.18 (S)	29.82 \pm 4.57 (HS)	21.89 \pm 6.01 (HS)	23.23 \pm 5.20 (HS)	18.32 \pm 4.78 (S)
Chuannong1A/HH1202	<i>xa7Xa7</i>	2.35 \pm 1.43 (R)	21.95 \pm 5.91 (HS)	2.01 \pm 1.96 (R)	2.30 \pm 4.03 (R)	0.31 \pm 0.17 (HR)	6.95 \pm 2.22 (MS)	19.80 \pm 4.46 (S)
Chuannong1A/HH1203	<i>xa7Xa7</i>	3.12 \pm 1.43 (R)	17.91 \pm 3.38 (S)	3.33 \pm 1.64 (MR)	0.80 \pm 0.33 (HR)	0.49 \pm 0.41 (HR)	5.78 \pm 2.48 (MS)	10.99 \pm 2.90 (MS)
Chuannong1A/HH1204	<i>xa21Xa21</i>	0.30 \pm 0.15 (HR)	6.22 \pm 1.73 (MS)	0.63 \pm 0.63 (HR)	5.94 \pm 2.08 (MS)	0.55 \pm 0.31 (HR)	23.71 \pm 4.83 (HS)	3.55 \pm 1.06 (MR)
Chuannong1A/HH1205	<i>xa21Xa21</i>	0.37 \pm 0.12 (HR)	5.54 \pm 1.81 (MS)	0.29 \pm 0.12 (HR)	5.31 \pm 0.11 (MS)	0.32 \pm 0.15 (HR)	15.70 \pm 2.84 (S)	2.78 \pm 1.24 (R)
Chuannong1A/HH1206	<i>xa7Xa7/xa21Xa21</i>	0.37 \pm 0.26 (HR)	4.90 \pm 1.89 (MR)	0.32 \pm 0.32 (HR)	0.57 \pm 0.28 (HR)	0.46 \pm 0.45 (HR)	7.43 \pm 1.99 (MS)	3.19 \pm 1.46 (MR)
Chuannong1A/HH1207	<i>xa7Xa7/xa21Xa21</i>	0.26 \pm 0.12 (HR)	4.80 \pm 1.87 (MR)	0.17 \pm 0.06 (HR)	0.64 \pm 0.27 (HR)	0.93 \pm 0.82 (HR)	5.10 \pm 1.61 (MS)	3.05 \pm 0.94 (R)

and South Asian rice growing countries. Broad resistance gene like *Xa21*, had successfully controlled the spread of rice BB in the world in the past two decades, but it was overcome by new virulent races in recent years in the Philippines, India, Korea and China (Marella et al., 2001; Lee et al., 1999; Zeng et al., 2002).

Therefore, it is necessary to identify and introduce new BB-resistance genes into rice breeding program besides finding tightly linked

molecular markers for MAS/MABC. Pyramiding resistance genes by MAS has been an effective method to control rice BB (Singh et al., 2001; Zhang et al., 2006; Loida et al., 2008; Gopalakrishnan et al., 2008).

In this study, *Xa7* and *Xa21* were selected and used for transferring them to popular restorer line on account of their being highly resistant against the most virulent and prevalent *Xoo* races, ZHE173 and GD1358 in the indica hybrid rice

growing area of Southern China (Zhang, 2009). Yihui1577 happens to be an elite restorer line and nearly seven hybrids had been released and planted in farmer fields over large areas in Southern China, but it was found to be susceptible to BB. In order to overcome the given constraint without changing much on agronomic and grain quality traits, we had selected six lines containing different BB-resistance genes by MABC approach. Evaluation of the six selected resistant lines and

Table 4. Disease reaction (average lesion length in cm \pm S.E.) and resistance levels of hybrids and male sterile lines upon inoculation with seven *Xoo* strains in 2010 at Wuhan.

Entry	Genotype of R gene	PX061	PX099	ZHE173	GD1358	YN24	FuJ	HEN11
JufengA	unknown	18.63 \pm 5.71 (S)	23.19 \pm 5.00 (HS)	17.03 \pm 3.80 (S)	12.81 \pm 1.61 (S)	17.88 \pm 4.18 (S)	24.35 \pm 2.85 (HS)	5.83 \pm 0.83 (MS)
JufengA/Yihui1577	unknown	8.20 \pm 2.25 (MS)	19.90 \pm 5.32 (S)	8.97 \pm 2.31 (S)	10.91 \pm 1.44 (MS)	15.93 \pm 3.43 (S)	28.05 \pm 5.33 (HS)	4.08 \pm 0.61 (MR)
JufengA/HH1202	<i>xa7Xa7</i>	1.88 \pm 0.78 (R)	18.57 \pm 2.62 (S)	0.92 \pm 0.77 (HR)	4.01 \pm 0.93 (MR)	11.96 \pm 4.51 (MS)	4.21 \pm 2.23 (MR)	4.37 \pm 1.10 (MR)
JufengA/HH1203	<i>xa7Xa7</i>	0.30 \pm 0.20 (HR)	21.21 \pm 6.67 (HS)	1.05 \pm 0.78 (R)	1.76 \pm 0.79 (R)	7.30 \pm 1.88 (MS)	4.80 \pm 1.75 (MR)	2.69 \pm 0.73 (R)
JufengA/HH1204	<i>xa21Xa21</i>	0.92 \pm 1.27 (HR)	14.80 \pm 3.55 (S)	2.01 \pm 1.47 (R)	2.22 \pm 0.84 (R)	6.75 \pm 2.61 (MS)	28.25 \pm 3.28 (HS)	1.75 \pm 0.40 (R)
JufengA/HH1205	<i>xa21Xa21</i>	0.26 \pm 0.22 (HR)	13.77 \pm 5.10 (S)	0.18 \pm 0.18 (HR)	0.68 \pm 0.38 (HR)	6.58 \pm 2.57 (MS)	28.21 \pm 2.88 (HS)	1.43 \pm 0.37 (R)
JufengA/HH1206	<i>xa7Xa7/xa21Xa21</i>	0.27 \pm 0.13 (HR)	17.09 \pm 6.65 (S)	0.16 \pm 0.15 (HR)	0.88 \pm 0.31 (HR)	5.69 \pm 1.87 (MS)	2.81 \pm 1.00 (R)	2.07 \pm 0.52 (R)
JufengA/HH1207	<i>xa7Xa7/xa21Xa21</i>	0.38 \pm 0.18 (HR)	17.74 \pm 5.27 (S)	0.19 \pm 0.19 (HR)	0.98 \pm 0.41 (HR)	5.52 \pm 1.14 (MS)	2.64 \pm 1.03 (R)	1.86 \pm 0.48 (R)
Chuan23A	unknown	17.26 \pm 3.87 (S)	26.82 \pm 6.58 (HS)	20.43 \pm 4.20 (HS)	13.75 \pm 2.04 (S)	17.46 \pm 3.14 (S)	25.14 \pm 4.35 (HS)	15.17 \pm 2.71 (S)
Chuan23A/Yihui1577	unknown	12.26 \pm 3.57 (S)	29.16 \pm 5.72 (HS)	11.05 \pm 2.82 (MS)	13.1 \pm 2.71 (S)	16.12 \pm 4.72 (S)	28.27 \pm 5.13 (HS)	6.46 \pm 1.39 (MS)
Chuan23A/HH1202	<i>xa7Xa7</i>	2.64 \pm 1.44 (R)	23.51 \pm 4.42 (HS)	1.24 \pm 1.01 (R)	4.00 \pm 1.13 (MR)	16.79 \pm 5.05 (S)	8.37 \pm 2.37 (MS)	6.84 \pm 1.34 (MS)
Chuan23A/HH1203	<i>xa7Xa7</i>	0.81 \pm 0.80 (HR)	28.23 \pm 6.25 (HS)	2.61 \pm 1.47 (R)	3.37 \pm 1.85 (MR)	18.28 \pm 5.28 (S)	6.53 \pm 2.03 (MS)	5.74 \pm 1.09 (MS)
Chuan23A/HH1204	<i>xa21Xa21</i>	1.31 \pm 0.92 (R)	13.70 \pm 3.98 (S)	1.11 \pm 1.12 (R)	1.53 \pm 0.62 (R)	7.26 \pm 2.07 (MS)	22.07 \pm 6.6 (HS)	2.85 \pm 0.61 (R)
Chuan23A/HH1205	<i>xa21Xa21</i>	0.78 \pm 0.64 (HR)	12.62 \pm 3.52 (S)	0.26 \pm 0.28 (HR)	1.14 \pm 0.44 (R)	8.96 \pm 2.71 (MS)	24.48 \pm 6.45 (HS)	1.61 \pm 0.33 (R)
Chuan23A/HH1206	<i>xa7Xa7/xa21Xa21</i>	0.30 \pm 0.37 (HR)	12.53 \pm 4.23 (S)	0.14 \pm 0.12 (HR)	1.24 \pm 0.39 (R)	8.36 \pm 2.65 (MS)	5.44 \pm 2.24 (MS)	2.52 \pm 0.37 (R)
Chuan23A/HH1207	<i>xa7Xa7/xa21Xa21</i>	0.83 \pm 0.64 (HR)	10.83 \pm 3.76 (MS)	0.21 \pm 0.21 (HR)	1.28 \pm 0.63 (R)	9.21 \pm 2.56 (MS)	6.27 \pm 2.25 (MS)	2.49 \pm 0.71 (R)

their derived hybrids showed that the different genotypes had different resistance spectrum of *Xoo* strains. These findings imply that we can use corresponding lines for deployment in different regions according to the distribution and prevalent degree of *Xoo* strains. Two selected resistant lines, HH1206 and HH1207 carrying two BB resistance genes *Xa7* and *Xa21* together and their derived hybrids had a stronger resistance level and much broader resistance spectrum than ILs carrying any one of the BB resistance gene individually. Therefore, it will be essential in the future to use pyramiding restorer lines with two or more *Xa* resistance genes in hybrid rice breeding program especially in continuous rice cropping regions where more *Xoo* strains are naturally available and BB diseases cause serious economic damage to rice farmers.

In hybrid rice program, two parents (female and

male) are involved in developing F₁ hybrids, and developing resistant hybrids using dominant BB resistance genes in any one parent should be sufficient. In this case, we had developed the male parent, that is, restorer line Yihui1577 to be introgressed with *Xa7* and *Xa21* genes by simple MABC approach with foreground selection scheme. It is expected that the F₁ should be equally resistant to the homozygous parental lines that contribute to it but we found that the susceptibility degree of female parents (male sterile lines) had a direct effect on the resistance degree of F₁ hybrids to *Xoo* strains. BB resistance levels and spectrum of F₁ hybrids with same male parents and different female parents were different from the same *Xoo* strains inoculated (Tables 3 and 4). This implies that it is necessary to introgress the BB-resistance genes into male sterile lines (female parents) while simultaneously

developing BB-resistance into male parents. It also suggests that the background effect of the hybrid in which the resistance genes are placed do matter. It may be due to several modifier genes that may influence the action of a given resistance gene. Recently, *Xa7* resistance levels was found be enhanced by high temperature 35/31°C (day/night) conditions (Webb et al., 2010). In general, foreground and background selections should be simultaneously carried out in backcrossing and MAS breeding program to maximize the gains. In this study, we had just made foreground and strong phenotypic selections in each backcross generation. This allowed us largely to achieve our objective of developing BB resistant Yihui1577 with *Xa7* and *Xa21* genes within three rounds of backcross as evident from the agronomic and grain quality traits evaluated. Similar findings have been reported by several researchers using MAS

Table 5. Agronomic performance and grain quality traits of the select improved BB resistant lines in comparison with recurrent parent.

Characteristic	Yihui1577	HH1202	HH1203	HH1204	HH1205	HH1206	HH1207
Days to 50% flowering (days)	97.33	96.00	97.33	96.67	97.33	96.33	97.00
Plant height (cm)	113.59	114.98	112.73	113.00	114.30	117.52	116.83
Productive panicles/plant	6.44	6.89	7.67	6.44	8.33**	7.44	6.56
Panicle length (cm)	22.03	21.66	21.61	22.58	21.23	21.37	21.04
spikelets/plant	1256.67	1217.78	1346.56	1368.53	1411.67	1532.33**	1368.22
Percent spikelet fertility (%)	79.71	83.63	82.71	76.45**	77.69	84.15	85.09
Yield/plant (g)	24.52	27.42*	27.87	25.15	26.86	29.37*	26.90
1000-grain weight (g)	24.70	26.94	25.03	24.06	24.53	22.80	23.12
Brown rice recovery rate (%)	74.70	75.57	74.81	73.92	74.24	76.41	75.21
Milled rice recovery rate (%)	63.35	62.92	61.22	62.58	62.48	63.59	63.25
Head rice recovery rate (%)	33.04	32.77	34.07	34.80	34.33	33.95	33.66
Chalkiness degree (%)	39.36	38.40	37.05	36.10	39.77	38.00	37.35
Length of milled head rice (mm)	7.00	6.96	6.90	6.65	6.58	6.75	6.82
Gel consistency (mm)	67.56	68.87	68.23	67.58	66.96	68.34	68.26
Alkali spreading value (score)	6	6	5	6	6	6	6
Amylose content (%)	20.98	21.13	20.36	22.82	21.79	20.77	21.35

Significant difference between the performance of Yihui1577 and six improved lines is indicated with single and double asterisk, *P = 0.05 and ** P = 0.01.

(Tian et al., 2011; Huang et al., 1997). Through this study, we have learnt that the foreground selection for markers can be cost effective and efficient provided we have a strong phenotypic selection which is kept in place primarily to identify segregants in each BC generation which is similar to the recurrent parent.

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Abbreviations

BB, Bacterial blight; **Xoo**, *Xanthomonas oryzae* pv. *oryzae*; **MABC**, marker-assisted backcross.

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