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Pyramiding of *Xa7* and *Xa21* for the improvement of disease resistance to bacterial blight in hybrid rice

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With 2 figures and 3 tables

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Abstract

'Minghui 63' is a restorer line widely used in hybrid rice production in China for the last two decades. This line and its derived hybrids, including 'Shanyou 63', are susceptible to bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). To improve the bacterial blight resistance of hybrid rice, two resistance genes *Xa21* and *Xa7*, have been introgressed into 'Minghui 63' by marker-assisted selection and conventional backcrossing, respectively. The single resistance gene-introgressed lines, Minghui 63 (*Xa21*) and Minghui 63 (*Xa7*) had higher levels of resistance to bacterial blight than their derived hybrids, Shanyou 63 (*Xa21*) or Shanyou 63 (*Xa7*). Both *Xa21* and *Xa7* showed incomplete dominance in the heterozygous background of rice hybrids by infection with GX325 and KS-1-21. The improved restorer lines, with the homozygous genotypes, *Xa21Xa21* or *Xa7Xa7*, were more resistant than their hybrids with the heterozygous genotypes *Xa21xa21* or *Xa7xa7*. To further enhance the bacterial blight resistance of 'Minghui 63' and its hybrids, *Xa21* and *Xa7* were pyramided into the same background using molecular marker-aided selection. The restorer lines developed with the resistance genes *Xa21* and *Xa7*, and their derived hybrids were evaluated for resistance after inoculation with 10 isolates of pathogens from China, Japan and the Philippines, and showed a higher level of resistance to BB than the restorer lines and derived hybrids having only one of the resistance genes. The pyramided double resistance lines and their derived hybrids have the same high level of resistance to BB. These results clearly indicate that pyramiding of dominant genes is a useful approach for improving BB resistance in hybrid rice.

Key words: *Oryza sativa* — bacterial blight — marker-assisted selection — resistance gene pyramiding

Hybrid rice has made a prominent contribution to rice production in China since it was first cultivated commercially in 1976, and hybrid rice production has expanded to more than 15.5 million ha annually. Hybrid rice, with a 20% yield advantage over inbred cultivars, has helped China produce 380 million tons more paddy rice from 1976 to 2002 (Ma and Yuan 2003). Hybrid rice has also been cultivated in several other Asian countries, and the planting area for hybrid rice is predicted to increase considerably in the years ahead.

Bacterial blight (BB) caused by *Xanthomonas oryzae oryzae* (*Xoo*) is one of the most destructive bacterial diseases in rice (Mew 1987). BB is also a serious problem in hybrid rice production because the widely used parental lines are susceptible to the pathogens under field conditions (Zhang et al. 1998).

A total of 27 major genes giving resistance to various strains of *X. oryzae oryzae* have so far been identified, referred to as *Xa1* to *Xa27* (Kinoshita 1994, 1995, Lin et al. 1996, Sonti 1998, Zhang et al. 1998, Chen et al. 2002, Gu et al. 2004). *Xa21* and *Xa7* are the two dominant genes providing resistance to *Xoo* races. *Xa21*, identified from the wild rice, *Oryza longistaminata*, is highly resistant to a broad spectrum of *Xoo* races. It was mapped on chromosome 11 and isolated by map-based cloning (Song et al. 1995). *Xa7* is another gene with broad-spectrum resistance and was originally identified in rice cultivar DV85 (Sidhu et al. 1978). Five molecular markers on chromosome 6 were shown to be linked to *Xa7*, using a near-isogenic F_3 population of IR24 × IRBB7 (Porter et al. 2003). To increase the resistance of hybrid rice to BB, *Xa21* was transferred into 'Minghui 63' from IRBB21 by marker-assisted selection (MAS) (Chen et al. 2000) and also by *Agrobacterium*-mediated gene transformation (Zhai et al. 2000). Another restorer line, Minghui 63 (*Xa7*), derived from DV85 and supposed to contain *Xa7*, was backcrossed to 'Minghui 63' several times (Ding 2005). These BB disease-resistant genes, however, have not been integrated into the same target plant of 'Minghui 63', thereby largely restricting their use in hybrid rice production.

Marker-assisted selection offers a unique opportunity to circumvent problems associated with phenotypic selection for traits of interest in conventional breeding programmes and it increases the breeding efficiency and flexibility by selecting for molecular markers linked to target genes or quantitative trait loci (QTL). Gene pyramiding is a very useful approach to utilize existing genetic resources. It has been successfully applied in several crop breeding programmes, leading to the development and/or release of many varieties and lines possessing multiple attributes (Huang et al. 1997, Wang et al. 2001, Samis et al. 2002, Jiang et al. 2004). Through gene interaction and complementation, lines with pyramided genes have been found to increase resistance quantitatively and to provide a wider spectrum of resistance over those conferred by single genes (Yoshimura et al. 1995, Singh et al. 2001).

In this study, a gene pyramiding process is reported that was used to combine two wide-spectrum BB resistance genes, *Xa21* and *Xa7*, into the same target plant of 'Minghui 63', an elite *indica* cytoplasmic male sterility (CMS) restorer line, through MAS. This aimed at further improving BB resistance of its derived hybrids, 'Shanyou 63'.

Materials and Methods

Plant materials: Two restorer lines, Minghui 63 (*Xa21*) and Minghui 63 (*Xa7*) of rice, *Oryza sativa* L., were used in this study. The former was bred from a repeated backcross procedure and MAS using IRBB21 as a donor and 'Minghui 63' as a recurrent parent (Chen et al. 2000). The latter is a BB-resistant line, which was derived from the original BB-resistant donor cultivar DV 85 and introgressed to 'Minghui 63' by conventional backcrossing. The original name of this line was Kanghui 63. Kanghui 63 was reported to carry *Xa7* (Ding 2005). It is referred to here as Minghui 63 (*Xa7*) because it has the same Minghui 63 background.

A cross between Minghui 63 (*Xa21*) and Minghui 63 (*Xa7*) was made in the autumn of 2000 in Wuhan, China. The F_1 seeds were obtained and sown in Hainan in the spring of 2001. The F_2 population and the $F_{2:3}$ families were planted in the normal rice-growing season in the experimental farm in 2001 and 2002, respectively, at Huazhong Agricultural University, Wuhan, China.

***Xoo* races, inoculum preparation, inoculation and disease scoring:** A total of 24 strains of *Xanthomonas oryzae oryzae* (*Xoo*) from China, the Philippines and Japan, kindly provided by Q. Zhang, L. Zhu, T. Mew and T. Ogawa were used to inoculate individual plants of 'Minghui 63', Minghui 63 (*Xa7*), Minghui 63 (*Xa21*), 'Shanyou 63' and its parents. Two *Xoo* isolates, KS-1-21 and GX325, were selected to inoculate individuals of the F_2 population derived from Minghui 63 (*Xa21*) × Minghui 63 (*Xa7*) for gene effect analysis. An $F_{2:3}$ population was selected from the F_2 against the *Xa21* gene via MAS in order to map the BB-resistant gene *Xa7* in Minghui 63 (*Xa7*). Ten *Xoo* isolates, which were selected for their high virulence to the original 'Minghui 63', were used to evaluate the pyramided lines and their derived hybrids (Table 3). The methods of isolate preparation and inoculation were the same as described previously (Lin et al. 1996). Disease scoring was performed following Chen et al. (2000). Plants were inoculated at the booting stage and the lesion length was scored 21 days later. Five leaves per plant and 10 plants per plot were scored. A plant was classified from its average lesion length as resistant (< 3.0 cm), moderately resistant (3.1–6.0 cm), moderately susceptible (6.1–9.0 cm), or susceptible (> 9.0 cm) (Chen et al. 2000).

Molecular marker assay: The experimental procedure for DNA isolation was essentially the same as previously described by Chen et al. (2000). The MAS system for positive selection of *Xa21* was according to Chen et al. (2000). One polymerase chain reaction (PCR)-based marker, RAPD248, which was a part of *Xa21*, was used for the selection of *Xa21* (Chunwongse et al. 1993). A further five PCR-based markers, M1, M2, M3, M4 and M5, which were closely linked to *Xa7* on chromosome 6, were kindly provided by Dr C. V. Cruz at IRRI, Manila, Philippines. The experimental procedure for PCR analysis of *Xa7* was the same as described by Porter et al. (2003).

Statistical analysis: Comparison of multiple mean values was performed by using the statistical software, JMP 5.0 (SAS Institute Inc.).

Results

Resistance spectrum of Minghui 63 (*Xa21*) and Minghui 63 (*Xa7*)

Twenty-four *Xoo* strains were used to inoculate 'Shanyou 63' and its parents, Zhenshan 97B (a maintainer line of Zhenshan 97A), 'Minghui 63', Minghui 63 (*Xa21*) and Minghui 63 (*Xa7*). Of the 24 *Xoo* strains tested, 'Shanyou 63' conferred a moderate or high level of susceptibility to 13 strains, and 'Minghui 63' and Zhenshan 97B were moderately or highly susceptible to 13 and 23 strains, respectively (Table 1). These results indicate that it would be useful and important to improve the BB resistance of this hybrid and its parents.

Both Minghui 63 (*Xa21*) and Minghui 63 (*Xa7*) were found to be susceptible to KS-1-21 and PXO99. Of the 24 *Xoo* stains tested, Minghui 63 (*Xa7*) conferred a high level of resistance to 20 strains, moderate resistance to two strains (GX325 and PXO71), moderate susceptibility to one strain (KS-1-21), and high susceptibility to one strain (PXO99). Minghui 63 (*Xa21*) conferred a high level of resistance to 17 strains, moderate resistance to five strains (I34, LN44, PXO112, PXO145 and PXO61), moderate susceptibility to one strain (KS-1-21) and

Table 1: Lesion length of 'Minghui 63', Minghui63 (*Xa21*), Minghui 63 (*Xa7*), Zhenshan 97 and 'Shanyou 63' after manual inoculation with *Xoo* strains in 2001 (Wuhan)

Strains	'Shanyou 63'	'Minghui 63'	Zhenshan 97B	Minghui 63 (*Xa21*)	Minghui 63 (*Xa7*)
FJ23	5.72 ± 0.98 (MR)[1]	5.28 ± 1.12 (MR)	9.23 ± 5.31 (S)	2.51 ± 0.62 (R)	2.21 ± 0.71 (R)
GD1358	4.57 ± 0.77 (MR)	3.87 ± 0.44 (MR)	5.51 ± 0.71 (MR)	1.39 ± 0.35 (R)	0.11 ± 0.0 (R)
GX325	22.61 ± 3.23 (S)	20.45 ± 1.70 (S)	19.20 ± 3.79 (S)	2.89 ± 1.25 (R)	3.19 ± 0.98 (MR)
HB-17	4.55 ± 1.10 (MR)	2.58 ± 0.95 (R)	6.81 ± 2.00 (MS)	0.82 ± 0.25 (R)	0.41 ± 0.51 (R)
OS105	2.18 ± 0.90 (R)	1.63 ± 0.62 (R)	10.57 ± 3.64 (S)	1.44 ± 1.47 (R)	0.10 ± 0.00 (R)
KS-1-21	29.21 ± 3.40 (S)	23.55 ± 2.48 (S)	22.76 ± 3.77 (S)	7.71 ± 1.56 (MS)	7.90 ± 1.77 (MS)
Zhe173	16.45 ± 2.87 (S)	19.43 ± 1.62 (S)	15.65 ± 2.38 (S)	2.18 ± 0.96 (R)	0.85 ± 0.56 (R)
JL691	1.24 ± 0.98 (R)	1.00 ± 0.83 (R)	22.00 ± 4.20 (S)	0.93 ± 0.90 (R)	1.58 ± 2.53 (R)
O249	11.68 ± 2.51 (S)	9.91 ± 1.76 (S)	13.09 ± 2.33 (S)	1.76 ± 0.76 (R)	0.10 ± 0.00 (R)
II247	5.43 ± 0.98 (MR)	5.43 ± 0.57 (MR)	6.18 ± 1.02 (MS)	2.72 ± 0.65 (R)	0.10 ± 0.00 (R)
I34	6.15 ± 0.93 (MS)	6.38 ± 1.12 (MS)	8.12 ± 0.88 (MS)	3.16 ± 0.77 (MR)	0.10 ± 0.00 (R)
II201	2.66 ± 1.69 (R)	2.54 ± 1.45 (R)	18.28 ± 4.75 (S)	0.76 ± 0.29 (R)	0.23 ± 0.48 (R)
II94	7.98 ± 1.85 (MS)	6.11 ± 1.39 (MS)	20.68 ± 4.21 (S)	1.50 ± 0.73 (R)	0.38 ± 0.40 (R)
LN44	19.00 ± 2.92 (S)	7.50 ± 0.89 (MS)	20.15 ± 4.30 (S)	4.15 ± 1.35 (MR)	0.20 ± 0.00 (R)
PXO280	9.03 ± 3.32 (S)	3.30 ± 0.78 (MR)	10.50 ± 3.13 (S)	1.87 ± 0.78 (R)	1.97 ± 0.87 (R)
PXO112	0.34 ± 0.15 (R)	2.93 ± 1.05 (R)	8.55 ± 3.02 (MS)	3.20 ± 1.11 (MR)	0.20 ± 0.00 (R)
PXO145	14.77 ± 2.23 (S)	13.18 ± 4.30 (S)	15.28 ± 2.32 (S)	4.65 ± 1.24 (MR)	2.72 ± 1.75 (R)
PXO79	6.81 ± 1.17 (MS)	3.34 ± 0.55 (MR)	13.28 ± 3.34 (S)	0.49 ± 0.25 (R)	0.15 ± 0.07 (R)
PXO61	11.69 ± 2.36 (S)	9.18 ± 2.74 (S)	27.80 ± 5.13 (S)	3.15 ± 1.55 (MR)	0.69 ± 0.54 (R)
PXO71	3.55 ± 2.38 (MR)	9.42 ± 2.42 (S)	24.75 ± 3.68 (S)	1.43 ± 0.61 (R)	4.35 ± 1.09 (MR)
PXO99	28.05 ± 2.37 (S)	29.00 ± 3.88 (S)	22.11 ± 2.88 (S)	10.44 ± 1.15 (S)	28.25 ± 3.52 (S)
PXO339	5.42 ± 1.03 (MR)	0.74 ± 0.26 (R)	21.70 ± 2.96 (S)	0.61 ± 0.21 (R)	1.18 ± 0.57 (R)
T7147	0.93 ± 0.39 (R)	6.05 ± 1.31 (MS)	15.78 ± 4.06 (S)	1.97 ± 0.81 (R)	0.29 ± 0.15 (R)
T7133	9.75 ± 1.54 (S)	8.45 ± 2.16 (MS)	20.05 ± 4.61 (S)	2.37 ± 0.81 (R)	0.10 ± 0.00 (R)

[1]R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.

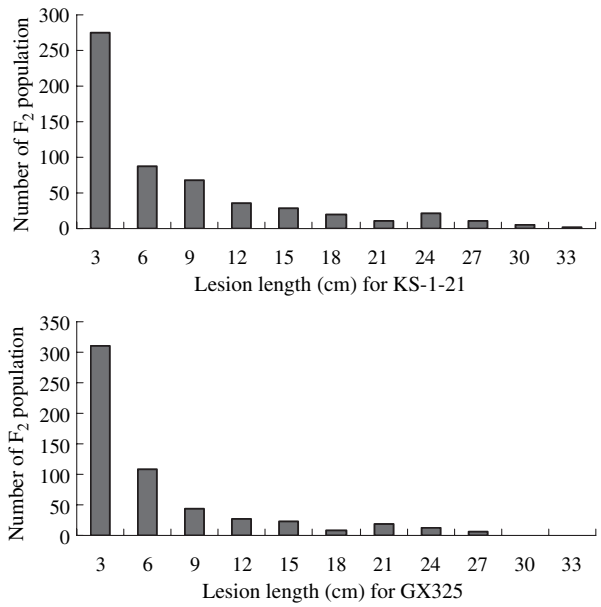

Fig. 1: The distribution of lesion length after inoculation with *Xoo* strain KS-1-21 and GX325, respectively, in 570 randomly selected individuals from an F_2 population derived from Minghui 63 (*Xa21*) and Minghui 63 (*Xa7*)

susceptibility to PXO99. Minghui 63 (*Xa7*) and Minghui 63 (*Xa21*) were both highly susceptible to PXO99. Both Minghui 63 (*Xa7*) and Minghui63 have the same lesion length (28–29 cm), with the lesion length of Minghui 63 (*Xa21*) being 10.44 cm. These results further indicate that Minghui 63 (*Xa7*) is completely susceptible to PXO99 while Minghui 63 (*Xa21*) has a slightly higher resistance to this race. It is therefore assumed that Minghui 63 (*Xa7*) carries a broad spectrum BB-resistance gene. Two strains, KS-1-21 and GX325, which showed intermediate virulence, and can be distinguished from those isolates virulent to *Xa21*, were selected to determine the genetic performance of BB resistance in F_2 and $F_{2:3}$ populations of a cross between Minghui 63 (*Xa7*) and Minghui 63 (*Xa21*).

BB resistance segregation in the $F_{2:3}$ population

As described in the Material and Methods section, 570 F_2 plants were obtained from the cross of Minghui 63 (*Xa7*) × Minghui 63 (*Xa21*). At the early tillering stage, different tillers of each F_2 plant were inoculated separately with two strains, KS-1-21 and GX325. The lesion lengths were measured and showed a bimodal distribution for the two strains tested (Fig. 1a,b). The segregation fits a 15 : 1 ratio when the plants are grouped with a lesion length of 18–20 cm as the cutting value (KS-1-21: $\chi^2 = 3.22$, $P > 0.05$; GX325: $\chi^2 = 0.01$, $P > 0.05$)

Gene effect analysis between *Xa21* and *Xa7* in the F_2 population

Two co-dominant PCR-based markers, RAPD 248 and M2, which marked the *Xa21* and *Xa7* loci, respectively, were used to genotype the 570 F_2 plants derived from the cross Minghui 63 (*Xa21*) × Minghui 63 (*Xa7*). These 570 plants were classified into nine genotypes determined by the two loci, *Xa21* and *Xa7*. Both isolates, KS-1-21 and GX325, were used to inoculate the F_2 population. The results are shown in Table 2. By comparing the multiple means for the nine genotypes, five distinct groups (A, B, C, D, E) were found for each isolate among these genotypes. The group A, *xa21xa21xa7xa7*, which has no resistance genes, was highly susceptible to BB with long lesion lengths (19.58 cm for GX325 and 22.07 cm for KS-1-21). For GX325, the genotypes that are homozygous at one of the loci, such as *Xa21Xa21xa7xa7* and *xa21xa21Xa7Xa7*, belong to the same group D and showed the same level of resistance. The genotypes that are heterozygous at one of the loci, such as *xa21xa21Xa7xa7* and *Xa21xa21xa7xa7*, belong to different groups (B and C) with a level of resistance between the control (*xa7xa7xa21xa21*) and the single-locus homozygote (*Xa7Xa7xa21xa21* and *xa7xa7Xa21Xa21*). These results show that both *Xa7* and *Xa21* are partially dominant, with the heterozygote *Xa21xa21* having a higher BB resistance than the heterozygote *Xa7xa7*. Genotypes responded a little differently to KS-1-21, than to GX325. The *Xa21*-homozygote, *xa7xa7Xa21Xa21*, showed stronger resistance than the *Xa7*-homozygote, *Xa7Xa7xa21xa21*. The *Xa*-21 heterozygous genotype, *xa7xa7Xa21xa21*, had the same resistance as the *Xa*-7 homozygote, *Xa7Xa7xa21xa21*, both of which belong to group C. For both GX325 and KS-1-21, the genotypes with at least one dominant allele at each locus, such as *Xa21xa21Xa7xa7*, *Xa21xa21Xa7Xa7*, *Xa21Xa21Xa7xa7* and *Xa21Xa21Xa7Xa7*, clearly belong to the same group E, and they showed higher resistance to BB than any of the other genotypes with dominant alleles at only one of the loci. It also showed that the combination of *Xa21* with *Xa7* created gene-additive effects for resistance to BB. Otherwise, the lesion length for BB resistance would have become gradually shorter with an increase in the numbers of dominant alleles, except for the double heterozygote genotypes, *Xa21xa21Xa7xa7*. It is obvious that BB resistance showed significant gene dosage effect by pyramiding of the genes .

Table 2: Comparison of bacterial resistance to GX325 and KS-1-21 as shown by lesion length among nine genotypes of an F_2 population using the Tukey–Kramer HSD test

Genotype[1]	No. of dominance allele	GX325					Mean	KS-1-21					Mean
xa21xa21xa7xa7	0	A[2]					19.58	A					22.07
xa21xa21Xa7xa7	1		B				12.38		B				14.20
Xa21xa21xa7xa7	1			C			6.47			C			7.96
Xa21Xa21xa7xa7	2				D		4.84				D		5.05
xa21xa21Xa7Xa7	2				D		4.31			C			7.16
Xa21Xa21Xa7xa7	3					E	2.67				D	E	3.64
Xa21xa21Xa7xa7	2					E	2.31					E	3.33
Xa21xa21Xa7Xa7	3					E	2.10					E	3.09
Xa21Xa21Xa7Xa7	4					E	1.61					E	2.23

[1]RAPD248 represents the *Xa21* gene locus and M2 the *Xa7* locus.
[2]Genotypes with same letters are not significantly different.

Table 3: Response of original and improved parental lines and their hybrids to *Xoo* isolates as shown by lesion length (cm) after inoculation at the booting stage (2003, Wuhan)

	PXO99	KS-1-21	GX325	PXO145	FJ23	ZHE173	PXO61	PXO71	T7133	LN44
Restorer line										
'Minghui 63'	22.35 ± 3.80 (S)[1]	21.68 ± 4.30 (S)	26.95 ± 3.63 (S)	12.18 ± 1.80 (S)	3.26 ± 0.22 (MR)	11.65 ± 2.72 (S)	15.25 ± 2.53 (S)	14.97 ± 3.45 (S)	18.37 ± 3.85 (S)	14.31 ± 5.13 (S)
Minghui 63 (*Xa7*)	23.46 ± 4.40 (S)	9.89 ± 3.69 (S)	4.21 ± 2.95 (MR)	0.76 ± 0.49 (R)	2.11 ± 1.00 (R)	0.80 ± 0.29 (R)	3.18 ± 1.41 (MR)	9.93 ± 2.30 (S)	0.55 ± 0.41 (R)	1.26 ± 0.63 (R)
Minghui 63 (*Xa21*)	14.13 ± 3.09 (S)	8.78 ± 2.41 (MS)	4.64 ± 2.48 (MR)	3.09 ± 1.66 (MR)	1.05 ± 0.36 (R)	2.30 ± 1.21 (R)	1.95 ± 0.67 (R)	2.32 ± 1.24 (R)	2.64 ± 1.81 (R)	3.98 ± 2.38 (MR)
Minghui63 (*Xa21*/*Xa7*)-1	15.45 ± 3.14 (S)	0.44 ± 0.14 (R)	0.40 ± 0.14 (R)	0.57 ± 0.31 (R)	0.47 ± 0.14 (R)	0.35 ± 0.20 (R)	0.50 ± 0.29 (R)	1.18 ± 0.57 (R)	0.45 ± 0.53 (R)	0.30 ± 0.08 (R)
Minghui63 (*Xa21*/*Xa7*)-2	14.27 ± 3.57 (S)	0.39 ± 0.11 (R)	0.34 ± 0.06 (R)	0.42 ± 0.13 (R)	0.44 ± 0.13 (R)	0.30 ± 0.08 (R)	0.34 ± 0.08 (R)	1.40 ± 0.30 (R)	0.26 ± 0.09 (R)	0.25 ± 0.10 (R)
Minghui63 (*Xa21*/*Xa7*)-3	13.93 ± 2.55 (S)	0.44 ± 0.16 (R)	0.37 ± 0.12 (R)	0.40 ± 0.07 (R)	0.43 ± 0.18 (R)	0.31 ± 0.08 (R)	0.33 ± 0.09 (R)	1.11 ± 0.36 (R)	0.26 ± 0.09 (R)	0.27 ± 0.10 (R)
Minghui63 (*Xa21*/*Xa7*)-4	16.14 ± 3.15 (S)	0.39 ± 0.07 (R)	0.31 ± 0.12 (R)	0.36 ± 0.09 (R)	0.35 ± 0.07 (R)	0.32 ± 0.07 (R)	0.30 ± 0.09 (R)	0.86 ± 0.73 (R)	0.22 ± 0.08 (R)	0.23 ± 0.07 (R)
Hybrid rice										
'Shanyou 63'	23.92 ± 1.48 (S)	23.26 ± 2.23 (S)	24.80 ± 2.69 (S)	17.88 ± 3.46 (S)	4.55 ± 0.36 (MR)	14.86 ± 2.25 (S)	15.67 ± 2.21 (S)	9.94 ± 4.22 (S)	20.63 ± 2.47 (S)	17.29 ± 2.15 (S)
Shanyou 63 (*Xa7*)	22.85 ± 1.48 (S)	18.44 ± 5.86 (S)	16.55 ± 2.46 (S)	3.58 ± 2.64 (MR)	3.15 ± 0.43 (MR)	2.24 ± 0.98 (R)	6.41 ± 1.65 (MS)	4.44 ± 1.62 (MR)	0.52 ± 0.16 (R)	1.62 ± 1.05 (R)
Shanyou 63 (*Xa21*)	17.03 ± 2.88 (S)	10.83 ± 1.36 (S)	8.38 ± 2.29 (MS)	4.31 ± 1.48 (MR)	2.41 ± 0.33 (R)	4.26 ± 0.83 (MR)	2.41 ± 0.87 (R)	1.35 ± 0.81 (R)	3.33 ± 1.87 (MR)	3.86 ± 1.15 (MR)
Shanyou 63 (*Xa21*/*Xa7*)-1	18.82 ± 2.07 (S)	0.78 ± 0.74 (R)	0.48 ± 0.34 (R)	1.14 ± 0.63 (R)	0.81 ± 0.19 (R)	0.36 ± 0.08 (R)	0.43 ± 0.11 (R)	0.50 ± 0.22 (R)	0.42 ± 0.12 (R)	0.24 ± 0.11 (R)
Shanyou63 (*Xa21*/*Xa7*)-2	17.51 ± 2.38 (S)	0.71 ± 0.49 (R)	0.47 ± 0.10 (R)	0.53 ± 0.19 (R)	0.96 ± 0.53 (R)	0.28 ± 0.08 (R)	0.38 ± 0.14 (R)	0.49 ± 0.23 (R)	0.43 ± 0.11 (R)	0.38 ± 0.13 (R)
Shanyou63 (*Xa21*/*Xa7*)-3	17.71 ± 1.19 (S)	0.46 ± 0.20 (R)	0.45 ± 0.10 (R)	0.76 ± 0.52 (R)	0.85 ± 0.16 (R)	0.35 ± 0.20 (R)	0.50 ± 0.24 (R)	0.44 ± 0.13 (R)	0.29 ± 0.08 (R)	0.51 ± 0.39 (R)
Shanyou 63 (*Xa21*/*Xa7*)-4	16.91 ± 1.13 (S)	0.45 ± 0.15 (R)	0.41 ± 0.21 (R)	0.47 ± 0.20 (R)	1.19 ± 0.77 (R)	0.32 ± 0.07 (R)	0.42 ± 0.19 (R)	0.77 ± 0.26 (R)	0.34 ± 0.17 (R)	0.46 ± 0.27 (R)

[1]R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.

Pyramiding *Xa21* and *Xa7* in the 'Minghui 63' background

From a large F_2 population derived from Minghui 63 (*Xa21*) and Minghui 63 (*Xa7*), a total of 172 plants, which were identified as being homozygous at *Xa21* by using RAPD248, were raised to an $F_{2:3}$ population. These *Xa21* homozygotes were then assayed using three markers, M2, M3 and M5, closely linked to *Xa7*. Thirty *Xa7* homozygous lines were obtained; these are assumed to be homozygous for both the *Xa21* and *Xa7* genes (Fig. 2a,b).

The gene effect of *Xa21* and *Xa7* in the background of 'Minghui 63' and its hybrids

Using the results listed in Table 1, 10 highly virulent pathogenic *Xoo* isolates to 'Minghui 63' were selected to inoculate 'Minghui 63', Minghui 63 (*Xa21*), Minghui 63 (*Xa7*) and the pyramided Minghui 63 (*Xa21*/*Xa7*) lines with two resistance genes at the booting stage (Table 3). 'Minghui 63' was highly susceptible to all the *Xoo* strains, except for FJ23 to which 'Minghui 63' was moderately resistant. Minghui 63 (*Xa7*) was highly susceptible to PXO99, KS-1-21 and PXO71, moderately resistant to GX325 and PXO61, and resistant to the remaining five strains. Minghui 63 (*Xa21*) was susceptible to PXO99, moderately susceptible to KS-1-21, moderately resistant to GX325, PXO145 and LN44, and resistant to the other six strains. These results showed that Minghui 63 (*Xa21*) and Minghui 63 (*Xa7*) were more resistant to BB than 'Minghui 63'. All the gene pyramided lines, with the genotype Minghui 63 (*Xa21*/*Xa7*), were highly resistant to *Xoo* strains, except for PXO99.

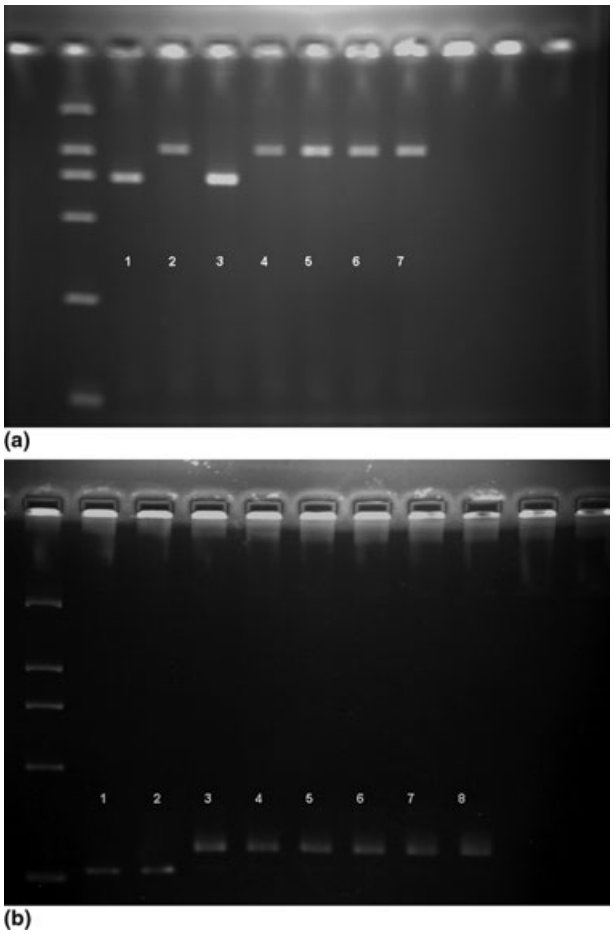

Fig. 2: Polymerase chain reaction analysis of 'Minghui 63', Minghui 63 (*Xa21*), Minghui 63 (*Xa7*) and its improved versions with both genes, *Xa21* and *Xa7*. (a) PCR analysis for *Xa21* with RAPD 248. (b) PCR analysis for *Xa7* with M3. In both (a) and (b), the samples of DNA are: 2-kb ladder; (1) 'Minghui 63'; (2) Minghui 63 (*Xa21*); (3) Minghui 63 (*Xa7*); (4) Minghui 63 (*Xa21*/*Xa7*)-1; (5) Minghui 63 (*Xa21*/*Xa7*)-2; (6) Minghui 63 (*Xa21*/*Xa7*)-3; (7) Minghui 63 (*Xa21*/*Xa7*)-4; (8) Minghui 63 (*Xa21*/*Xa7*)-5. Results in (a) and (b) confirm *Xa21* and *Xa7* genes in the pyramiding lines

The 10 *Xoo* strains were also used to inoculate the hybrids 'Shanyou 63', Shanyou 63 (*Xa7*), Shanyou 63 (*Xa21*), and the pyramided line, Shanyou 63 (*Xa21*/*Xa7*) at the booting stage (Table 3). 'Shanyou 63' conferred moderate resistance to one strain (FJ23), but a high level of susceptibility to the other nine strains (PXO99, KS-1-21, GX325, PXO145, ZHE173, PXO61, POX71, T7133 and LN44). Shanyou 63 (*Xa7*) conferred a high level of susceptible to three strains (PXO99, KS-1-21 and GX325) and moderately susceptibility to one strain (PX061), while Shanyou 63 (*Xa21*) conferred a high level of susceptibility to two strains (PXO99 and KS-1-21), and moderate susceptibility to one strain (GX325). Both Shanyou 63 (*Xa7*) and Shanyou 63 (*Xa21*) showed resistance or moderate resistance to the rest of the *Xoo* strains. All the pyramided hybrids, Shanyou 63 (*Xa21*/*Xa7*), showed a high level of resistance to all *Xoo* strains, except for PXO99. The gene-pyramided restorer line and its derived hybrids showed the same strong resistance to BB (Table 3). These results indicated that gene pyramiding could improve BB resistance and broaden the resistance spectrum of hybrid rice.

Discussion

'Minghui 63' is one of the best of the restorer lines for a number of commonly used hybrids in China, including 'Shanyou 63', the most widely cultivated hybrid in central and southern China for the last two decades. The results presented in this study showed that two resistance genes, *Xa21* and *Xa7*, have been successfully pyramided by MAS into the same plant of the elite *indica* CMS restorer line, 'Minghui 63'. The resulting parental plants and their derived hybrids showed a high level of resistance to 10 *Xoo* strains. The levels of resistance in the pyramided (double resistance) lines and their derived hybrids were much higher than those of the single resistance gene hosts, as reported previously (Chen et al. 2000, Jiang et al. 2004).

Gene pyramiding has been used to improve BB resistance. Huang et al. (1997) and Singh et al. (2001) attempted to pyramid dominant or recessive BB-resistant genes to improve conventional inbred varieties. Recessive genes are not suitable for improving hybrid rice because they can only be expressed when the alleles are homozygous. Dominant genes were found to be highly efficient for the improvement of resistance and other favourable agronomic traits in hybrid rice. Two dominant genes, *Xa21* and *Bt*, were first pyramided to the hybrid 'Shanyou 63' to confer disease and insect resistance. The resulting plants and their derived hybrids showed high levels of resistance to natural infestations of leaf fodders and yellow stem borers. Based on resistance to *Xoo* strains when manually inoculated, the restorer lines were more resistant than its derived hybrids (Jiang et al. 2004). To further improve BB resistance for hybrid rice, another broad-spectrum resistance gene, *Xa7*, was introgressed into the Minghui 63 (*Xa21*) background in this study. In the F_2 population between

Minghui 63 (*Xa21*)/Minghui 63 (*Xa7*), *Xa21* and *Xa7* showed significant interaction and dosage effects. Homozygous dominant genotypes, such as *Xa21Xa21* or *Xa7Xa7*, had significantly stronger resistance to BB than heterozygous genotypes such as *Xa21xa21* or *Xa7xa7*, while the latter had stronger resistance than homozygous recessive genotypes. The dosage effects indicate that both *Xa21* and *Xa7* provide incompletely dominant resistance to BB by inoculating with GX325 and KS-1-21. The results also indicated that the partial dominance of these genes was responsible for the decreased BB resistance that was found in hybrid rice. Combing for two incompletely dominant genes is a practical strategy to improve BB resistance in hybrid rice breeding. This research shows that plants containing both the *Xa21* and *Xa7* genes have the same strong resistance regardless of whether they are heterozygous or homozygous. The pyramided lines with both *Xa21* and *Xa7* are more resistant to BB than other genotypes that have only one resistance gene. Not only has resistance to BB been increased but the spectrum of resistance has also been widened when *Xa21* and *Xa7* were combined, indicating that there is an interaction additive effect between these two genes. This is very useful in hybrid rice breeding. The pyramided lines developed in this study should be used immediately for the development of high BB resistance in hybrid rice.

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