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Improvement of Bacterial Blight Resistance of 'Minghui 63', an Elite Restorer Line of Hybrid Rice, by Molecular Marker-Assisted Selection

Sheng Chen, X. H. Lin, C. G. Xu, and Qifa Zhang*

ABSTRACT

'Minghui 63' is a restorer line widely used in hybrid rice production in China. However, this line has become increasingly susceptible to bacterial blight (BB), resulting in a rapid decline of its use in rice production. The objective of this study was to improve the BB resistance of Minghui 63 by introgressing *Xa21*, a broad-spectrum BB resistance gene, into Minghui 63 by molecular marker-assisted selection (MAS). A polymerase chain reaction (PCR)-based MAS system was established consisting of a marker that is a part of *Xa21*, a marker located at 0.8 centimorgans (cM) from the *Xa21* locus on one side, and a marker at 3.0 cM from the gene on the other side. A total of 128 restriction fragment length polymorphism (RFLP) markers, evenly distributed on the 12 chromosomes, were used to recover the genetic background of Minghui 63. The resulting improved version of Minghui 63, or 'Minghui 63(*Xa21*)', was exactly the same as the original except for a fragment of less than 3.8 cM in length surrounding the *Xa21* locus. Both Minghui 63(*Xa21*) and its hybrid with 'Zhenshan 97A' referred to as 'Shanyou 63(*Xa21*)' showed the same spectrum of BB resistance as the donor parent. Field examination of a number of agronomic traits showed that Minghui 63(*Xa21*) and Shanyou 63(*Xa21*) were identical to Minghui 63 and Shanyou 63, when there was no disease stress. Under heavily diseased conditions, Minghui 63(*Xa21*) showed significantly higher grain weight and spikelet fertility than Minghui 63, and Shanyou 63(*Xa21*) was significantly higher than Shanyou 63 in grains per panicle, grain weight, and yield.

MINGHUI 63 is the restorer line for a number of widely used hybrids in rice production in China. The general characteristics of the hybrids produced with this restorer line include high yield and wide adaptability, which enabled these hybrids to occupy a total area of approximately 7 million hectares per year in the late 1980s and early 1990s, accounting for more than 25% of the total rice production area in China during that period. The most prominent example of these hybrids is 'Shanyou 63', a cross between the male sterile line 'Zhenshan 97A' and Minghui 63, that by itself accounted for a total area of 6.7 million hectares per year in its peak period. Clearly, this line has made a major contribution to the success of hybrid rice in China.

In recent years, however, there has been a rapid decline of the area planted to these hybrids. One of the main reasons for this reduction of production area is the breakdown of resistance to bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), one of the most destructive diseases of rice worldwide (Mew, 1987). It is known that Minghui 63 carries the BB resistance gene *Xa4* (Y. F. Tan, unpublished data), and was considered to be BB resistant when it was first released. However, its resistance has largely become ineffective during the period of extensive cultivation as a result of evolution of the pathogen population.

A large number of genes for BB resistance have been identified that are available for cultivar improvement (Ogawa et al., 1989; Khush et al., 1990; Lin et al., 1996). However, it has been difficult to use these genes to improve the resistance of the parents for the purpose of hybrid improvement. Incorporation of a resistance gene is difficult with conventional breeding methods because of linkage with undesirable traits that is very difficult to break even with many generations of backcrosses (Young and Tanksley, 1989).

Marker-assisted selection (MAS) has been advocated as a highly efficient breeding method, because it can offer rapid and precise selection of the targeted gene (Tanksley et al., 1989). Recent developments in genome research have provided a large number of molecular markers in many crop species and also diverse techniques for detection, which have made MAS a reality for application in breeding programs. In rice, for example, there have been studies demonstrating the feasibility of using MAS to pyramid genes for BB resistance (Yoshimura et al., 1995; Huang et al., 1997).

The objectives of the study reported in this paper were to improve the BB resistance of Minghui 63 by introgressing *Xa21*, a gene that is highly resistant to a broad spectrum of the pathogen races (Khush et al., 1990), by means of MAS in the process of recurrent backcrossing; and to evaluate the effects of such improvement on the agronomic performance of Minghui 63 and the hybrid under both BB stressed and nonstressed conditions.

MATERIALS AND METHODS

Cultivars, Combinations, and Populations

The plant materials used in this study included four indica (*Oryza sativa* ssp. *indica*) cultivars (or lines): (i) 'IRBB21', an isogenic line of 'IR24' containing *Xa21* (Ikeda et al., 1991) kindly provided by the International Rice Research Institute, was used as the donor parent of *Xa21*; (ii) Minghui 63, the restorer line for a number of elite hybrids widely grown in China, was the recipient parent to be improved; (iii) Zhenshan 97A, the male sterile line for a number of widely grown hybrids, was used to make hybrids with Minghui 63 as well as the improved version of Minghui 63; and (iv) 'Zhenzhuai', a cultivar highly susceptible to all the *Xoo* races, was used as a susceptible check.

Xoo Strains, Inoculum Preparation, Inoculation, and Disease Scoring

A total of 17 *Xoo* strains were used in this study (see Table 1 for representative examples), including nine strains from

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Abbreviations: BB, bacterial blight; cM, centimorgan; MAS, marker-assisted selection; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; *Xoo*, *Xanthomonas oryzae* pv. *oryzae*.

Table 1. Reactions of six rice lines to six representative examples of the 17 *Xanthomonas oryzac* pv. *oryzae* (*Xoo*) strains used for testing BB resistance.

Xoo pathogen			Disease score†					
Strain	Origin	Racial group‡	Minghui 63	Zhenshan 97	Shanyou 63	IRBB21	Zhenshan 97/IRBB21	Zhenzhuai
72-67	Hunan, China		S	S	S	MR	MR	S
KS6-6	Jiangsu, China	2	MS	S	S	R	R	S
LN44	Liaoning, China	1	MS	S	S	R	R	S
ZJ173	Zhejiang, China	4	S	S	S	R	R	S
Pxo99	Philippines	6	S	S	S	R	R	S
T7147	Japan	2	MS	S	S	S	S	S

† R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.
‡ The racial groups of the strains from China were based on Fang et al. (1990), the racial groups of strains from the Philippines were according to Mew (1987), and the racial groups of the strains from Japan followed those of Horino (1978). The same numeric code for racial groups of different countries does not mean the same race.

China kindly provided by Q. Zhang and L. Zhu, five strains from the Philippines provided by T.W. Mew, and three strains from Japan provided by T. Ogawa. The methods of inoculum preparation and inoculation were the same as described previously (Lin et al., 1996). The evaluation of BB resistance of the breeding materials was conducted in the disease nursery of Huazhong Agricultural University, Wuhan, China. For disease scoring, the length of the longest lesions of five undamaged leaves of each individual was measured 21 d after inoculation. A plant was classified as resistant if the average lesion length was shorter than 3.0 cm, moderately resistant if the lesion was 3.0 to 6.0 cm, moderately susceptible if the lesion was 6.0 to 9.0 cm, and susceptible if the lesion was longer than 9.0 cm.

RFLP and PCR Markers Used for MAS

To establish a MAS system for introgressing *Xa21* from IRBB21 to Minghui 63, 200 individuals from the F_2 population of a cross between these two lines were assayed individually with 11 markers (see Fig. 1) surrounding the *Xa21* genomic region. Four of the markers, 248, 21, C189 (converted from RFLP), and AB9 were PCR markers, and the remaining were RFLP markers selected from two high density maps of Causse et al. (1994) and Kurata et al. (1994). RG103 and 21 were sequences that are parts of the *Xa21* gene (Song et al., 1995). A local linkage map for this genomic region was constructed with Mapmaker/Exp 3.0 (Lincoln et al., 1992) with LOD 3.0. Cosegregation was also tested between resistance and the marker representing the *Xa21* locus by inoculating this population with *Xoo* strain Pxo99.

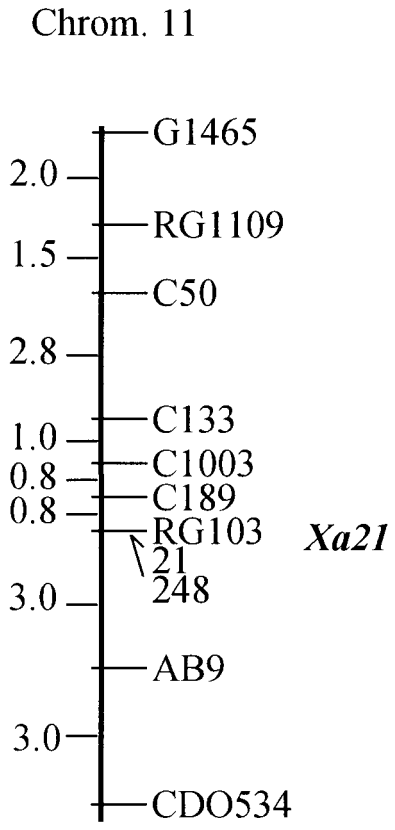

Fig. 1. The linkage map of the *Xa21* genomic region on rice chromosome 11.

The primers for three of the PCR markers, 248 (forward: 5′-AGA CGC GGA AGG GTG GTT CCC GGA-3′, reverse: 5′-AGA CGC GGT AAT CGA AAG ATG AAA-3′), 21 (forward: 5′-ATA GCA ACT GAT TGC TTG G- 3′, reverse: 5′-CGA TCG GTA TAA CAG CAA AAC-3′) and AB9 (forward: 5′-GGG CGA CTA CTA CAA AAC AT-3′, reverse: 5′-GGG CGA CTA CAG AGT TCA-3′), were from previously published sequences (Chunwongse et al., 1993; Wang et al., 1996; Williams et al., 1996). For C189, the forward primer (5′-AAG AAG TTG GAG CAG CAG GA-3′) was based on the DNA sequence of the RFLP probe C189 in DDBJ (DNA Data Bank of Japan) and the reverse primer was a 10-base random sequence (5′-CCG CAG TCT G-3′). AB9 is a dominant marker with the PCR fragment from the donor parent IRBB21 detected in the breeding population, and the remaining three markers are codominant.

The experimental procedures for RFLP assays, including DNA isolation, digestion, electrophoresis, and southern blot hybridization, were done essentially as described previously (Liu et al., 1997). DNA for PCR analysis was isolated according to the method of K.L. Zheng et al. (personal communication). The PCR analysis was conducted essentially according to Williams et al. (1996) except that the annealing temperature was lowered to 40°C for C189 that contained a 10-base primer.

The Crossing and Selection Scheme

The *Xa21* gene was introgressed into Minghui 63 following a recurrent backcrossing procedure, combined with tandem selection using molecular markers. The entire scheme took three generations of backcrosses and one generation of selfing to complete. In this scheme, the progeny of each backcross was first selected for the presence of the *Xa21* gene by means of both PCR and disease inoculation. The *Xa21*-containing individuals in the BC_1F_1 were selected for recombination between *Xa21* and either of the flanking marker loci. In BC_2F_1, the *Xa21*-containing individuals were selected for recombination between *Xa21* and the other marker locus. The *Xa21*-containing plants in the BC_3F_1 were assayed with a large number of molecular markers covering the entire rice genome to identify individuals that were homozygous for the Minghui 63

genotypes at all marker loci, except the *Xa21* locus. The selected individuals were then self-fertilized to produce individuals that were homozygous for the *Xa21* gene at this locus, thus completing the breeding procedure.

Collection of Field Data for Agronomic Traits

Agronomic performance of Minghui 63, Shanyou 63, and the *Xa21*-containing versions of Minghui 63 and Shanyou 63 was compared in Wuhan in the summer of 1998 and in Hainan (South China Sea) Island in the spring of 1999. In the Wuhan test, all the eight lines that were obtained in the BC_3F_2 generation (see the Results section) were planted along with the original Minghui 63 and IRBB21 in a field without artificial inoculation. Shanyou 63 and the *Xa21*-containing version of Shanyou 63 were planted in the disease nursery in two plots. Plants in one of the two plots were inoculated with ZJ173, a prevalent *Xoo* strain in rice growing areas of central and southern China, to produce heavily diseased conditions, while the uninoculated plots did not have much disease under natural conditions. In all the cases, each of the plots consisted of three rows with nine plants per row at planting density of 17 cm between plants in a row, and the rows were 27 cm apart. Only the five plants in the middle of the center row were used for measuring the agronomic traits.

In the Hainan planting, all the materials were tested in replicated field trials both under heavily diseased conditions and under natural field conditions that did not show much disease. For testing under heavily diseased conditions, Minghui 63, Shanyou 63, IRBB21, and the *Xa21*-containing versions of Minghui 63 and Shanyou 63 were each planted in a five-row plot, with ten plants per row. The distance between plants within a row was 10 cm and the rows were 20 cm apart. The three rows in the middle of each plot were inoculated with *Xoo* strains ZJ173, Pxo99, and LN44, respectively. Each of the plots was replicated twice, and the layout of the plots in the field was completely at random.

For testing under natural field conditions without much disease, the same set of materials was planted in the field without artificial inoculation. The sizes and the layout of the plots were the same as described in the previous paragraph except that the plantings followed a randomized complete block design with two replications.

Measurements were taken for the eight plants in the middle of three central rows (24 plants in total) in each plot for a number of agronomic traits, including heading date, plant height, tillers per plant, number of grains per panicle, weight of 1000 grains, and grain yield per plant.

RESULTS

Resistance of the Parents and Their Hybrids

To determine the potential usefulness of *Xa21* in rice hybrids, we inoculated Minghui 63, IRBB21, Zhenshan 97A, and their hybrids Zhenshan 97A/Minghui 63 (Shanyou 63) and Zhenshan 97A/IRBB21 in the disease nursery with all the 17 *Xoo* strains (data for representative examples are given in Table 1). Minghui 63 was moderately susceptible to most of the strains tested, and Shanyou 63 was more susceptible than Minghui 63 to all the strains that could attack Minghui 63. IRBB21 was resistant to all strains except T7147, a strain from Japan belonging to the Japanese racial group 2, which confirmed the previous results that *Xa21* is highly resistant to a broad racial spectrum of the pathogen (Khush et al., 1990). The resistance reaction of the hybrid Zhenshan 97A/IRBB21 appeared to be exactly the same as that of IRBB21, indicating a complete dominance of the resistance conferred by the gene(s) of IRBB21. Thus, *Xa21* would be very useful for improving BB resistance of the hybrid Shanyou 63.

MAS

The local linkage map of the *Xa21* genomic region on chromosome 11, constructed on the basis of 200 individuals of the F_2 population from the Minghui 63/IRBB21 cross, is shown in Fig. 1, from which a MAS system was established. Two markers, 21 and 248, cosegregated with the *Xa21* locus. These two markers were used for positive selection, i.e., selecting for the presence of the *Xa21* gene. Two additional markers, C189 and AB9, flanked both sides of the *Xa21* locus at 0.8 and 3.0 cM, respectively. These two markers were chosen for negative selection, i.e., selecting for recombination between the *Xa21* locus and the flanking markers. Such negative selection would ensure that the introgressed segment surrounding the *Xa21* locus was shorter than the length between the two flanking markers (3.8 cM).

The molecular marker-assisted backcross breeding was conducted with the above MAS system. Among a total of 49 plants in BC_1F_1 that contained *Xa21* as determined by both disease inoculation and PCR selection, one individual was found to be a recombinant between *Xa21* and the marker locus AB9 and was subsequently backcrossed to Minghui 63. In the same way, one of the 180 *Xa21*-containing plants in BC_2F_1 was found to be a recombinant between *Xa21* and the marker locus C189. Thus an individual containing an introgressed segment of less than 3.8 cM in length (0.21% of the rice genome, assuming a total length of 1800 cM) was obtained in BC_2F_1, and was further backcrossed to Minghui 63 to obtain the BC_3F_1.

Screening of 354 RFLP probes from the two published high-density maps (Causse et al., 1994; Kurata et al., 1994) identified 158 markers that were polymorphic between Minghui 63 and IRBB21 (Table 2). These markers were distributed quite evenly in the linkage map except that there were more markers from chromosome 11 than from the other chromosomes. One hundred

Table 2. Screening for RFLP markers that were polymorphic between Minghui 63 and IRBB21.

Chromosome	Markers screened	Polymorphic markers	Ratio of polymorphism
	no.		%
1	44	11	25
2	24	13	54
3	21	15	71
4	30	11	37
5	28	10	36
6	25	10	40
7	37	13	35
8	24	11	46
9	23	9	39
10	34	11	32
11	45	31	69
12	19	13	68
Total	354	158	45

Table 3. The molecular marker genotypes and resistance of eight selected individuals from 250 BC_3F_1 resistant plants. The molecular marker genotypes are based on 128 RFLP loci that were polymorphic between Minghui 63 and IRBB21 and evenly distributed on the 12 rice chromosomes.

Plant number	Number of heterozygous loci†	Heterozygous locus–chromosome	Lesion length‡
			cm
31-1	1	RG103/11	3.5 ± 1.5
31.2	1	RG103/11	2.5 ± 1.2
31.3	2	RG634/7, RG103/11	3.0 ± 0.8
31-4	3	RG151/2, C513/4, RG103/11	3.0 ± 1.5
31-5	4	C513/4, RG634/7, RZ900/11, RG103/11	2.5 ± 1.2
31-6	4	C513/4, RG634/7, RZ900/11, RG103/11	3.0 ± 1.3
31-7	5	RG151/2, RG256/2, C513/4, RZ900/11, RG103/11	3.0 ± 0.7
31-8	5	RZ489/1, C513/4, RG634/7, RZ900/11, RG103/11	2.5 ± 0.9
Minghui 63			18.5 ± 8.8
IRBB21			2.2 ± 1.2

† All the remaining marker loci were homozygous for the Minghui 63 genotypes.
‡ Average lesion length of 5 inoculated leaves at 21 d after inoculation with Pxo99.

twenty-eight of the 158 polymorphic markers, representing all the 12 chromosomes with the largest interval less than 30 cM, were used for ''cleaning up'' the genetic background of the selections. Among a total of 250 plants that carried *Xa21* in BC_3F_1, two plants were found to be homozygous for the Minghui 63 genotypes at all marker loci except the RG103 locus residing in the *Xa21* gene region. There were six additional plants that were heterozygous at one to four marker loci besides the RG103 locus. The lesion lengths of these eight plants were not significantly different from that of IRBB21 when tested against Pxo99, a strain belonging to *Xoo* Race 6 of the Philippines (Table 3).

These eight plants were self-pollinated to produce BC_3F_2, from which progenies homozygous for the *Xa21* allele were obtained. Compared with the original Minghui 63, the BB resistance of these selected lines was greatly improved according to the results of inoculations with a total of 12 *Xoo* strains (see Table 4 for the results from some of the strains). Such improved resistance was achieved without causing obvious differences in the agronomic traits between Minghui 63 and the improved lines under field conditions without artificial inoculation (Wuhan planting in the summer of 1998, data not shown). Thus, the two lines, that were homozygous for the *Xa21* allele and also homozygous for the Minghui 63 genotypes at all the marker loci, were selected and designated as 'Minghui 63(*Xa21*)'. One of the Minghui 63(*Xa21*) lines (32-2) was crossed with Zhenshan 97A to produce a hybrid, tentatively designated as 'Shanyou 63(*Xa21*)'.

Comparison of the Agronomic Performance

The results from the Wuhan planting in the summer of 1998 (data not shown) showed that yield and yield component traits were almost identical between the original Shanyou 63 and Shanyou 63(*Xa21*) when there was no artificial inoculation. On the other hand, the measurements for yield and two of the yield component traits (grains per panicle and grain weight) of Shanyou 63(*Xa21*) appeared to be higher than Shanyou 63 when challenged with the disease. However, because of the small numbers of plants available for the comparison because of the limited seed supply at the time when the experiment was performed, the data did not allow a rigorous statistical test.

In the Hainan test without artificial inoculation, Minghui 63 and Minghui 63(*Xa21*) were identical for all the agronomic traits examined, as were Shanyou 63 and Shanyou 63(*Xa21*) (Table 5). Under heavily diseased conditions (Table 5), Minghui 63(*Xa21*) showed significantly higher grain weight and spikelet fertility than Minghui 63. The differences in yield related traits were even more pronounced between Shanyou 63 and Shanyou 63(*Xa21*) (Table 5). Yield and all three yield component traits (tillers per plant, grains per panicle, and grain weight) were higher for Shanyou 63(*Xa21*) than Shanyou 63.

Table 4. Examples of BB resistance of eight selected lines from BC_3F_2, expressed as average lesion length ± SD when inoculated with six isolates of the pathogen.

Line–cultivar	Lesion length of isolate					
	76-27	KS6-6	LN44	ZJ173	Pxo99	T7147
	cm					
32-1	3.5 ± 1.2	2.3 ± 1.2	1.6 ± 1.5	2.5 ± 1.2	2.4 ± 1.2	8.5 ± 2.4
32-2	4.0 ± 1.5	1.5 ± 0.6	0.7 ± 0.5	1.0 ± 1.0	1.5 ± 1.2	8.8 ± 3.2
32-3	5.0 ± 2.5	2.6 ± 1.2	2.5 ± 0.5	2.0 ± 1.5	1.2 ± 0.8	7.6 ± 3.5
32-4	5.4 ± 2.3	2.4 ± 1.4	2.6 ± 1.2	2.5 ± 1.3	3.8 ± 2.0	7.6 ± 2.4
32-5	4.5 ± 2.0	2.2 ± 1.0	2.4 ± 1.0	1.8 ± 1.0	3.5 ± 1.8	7.5 ± 3.4
32-6	5.0 ± 1.2	2.5 ± 1.2	1.9 ± 1.2	1.5 ± 1.2	2.4 ± 1.5	8.6 ± 3.5
32-7	4.8 ± 2.0	2.5 ± 0.8	1.1 ± 0.6	1.8 ± 0.8	2.0 ± 1.4	9.8 ± 2.4
32-8	5.5 ± 1.2	1.8 ± 1.0	1.5 ± 0.8	2.5 ± 1.4	2.2 ± 1.2	7.2 ± 2.4
Minghui 63	11.8 ± 2.6	6.9 ± 2.7	7.5 ± 3.1	21.0 ± 4.3	14.5 ± 4.5	7.8 ± 2.5
IRBB21	4.2 ± 1.2	1.6 ± 1.5	1.5 ± 0.9	1.0 ± 0.5	2.2 ± 0.6	12.2 ± 2.8
Zhenzhuai	27.4 ± 4.5	17.3 ± 4.0	17.6 ± 2.4	23.2 ± 3.8	15.9 ± 2.2	15.4 ± 2.4

Table 5. Agronomic performance of Minghui 63, Shanyou 63, IRBB21 and *Xa21* containing versions of Minghui 63 and Shanyou 63 under field conditions with or without artificial inoculation tested in the spring of 1999 in Hainan Island.

	Days to heading	Plant height	Panicle length	Tillers–plant	Grains–panicle	Grain weight	Yield–plant	Spikelet fertility
	days	cm		no.		g/1000	g	%
Without inoculation								
Minghui 63	116.7	76.2	22.7	5.1	73.5	28.04	10.45	69.12
Minghui 63 (*Xa21*)	116.9	76.4	23.0	5.2	74.1	28.25	10.79	68.94
IRBB21	110.5	64.7	20.2	5.2	75.1	19.95	7.80	74.44
Shanyou 63	100.8	81.2	24.4	5.2	92.2	28.30	13.55	78.34
Shanyou 63 (*XA21*)	100.3	81.4	24.3	5.2	92.2	29.08	13.98	80.42
LSD (0.05)†	0.9	3.1	1.9	ns	7.4	1.12	1.97	4.99
LSD (0.01)‡	1.6	5.1	3.2	ns	12.3	1.86	3.27	8.27
Inoculated with strains Pxo99, ZJ173 and LN44								
Minghui 63	117.5	77.5	22.9	5.3	68.6	26.12	9.52	59.28
Minghui 63 (*Xa21*)	116.9	76.7	23.1	4.8	73.2	27.97	9.81	66.90
IRBB21	110.5	64.9	20.4	5.4	73.1	20.14	7.96	74.67
Shanyou 63	100.1	82.8	23.6	4.8	74.6	26.89	9.52	81.63
Shanyou 63 (*XA21*)	100.3	80.9	23.7	5.2	91.1	28.80	13.56	79.36
LSD (0.05)†	1.7	2.8	2.0	ns	12.2	1.21	1.09	5.45
LSD (0.01)‡	2.8	4.6	3.3	ns	20.2	2.01	1.80	9.03

†, ‡ Least significant differences at 0.05 and 0.01 probability levels, respectively.

DISCUSSION

Using MAS and three generations of backcrosses followed by one generation of selfing, we obtained an improved version of Minghui 63, the best restorer line widely used in rice production in China. We showed that this improved version of Minghui 63 contained only a fragment of less than 3.8 cM (<0.21%) in length surrounding the *Xa21* locus from the donor parent, with the rest of the genome exactly the same as the recipient parent. We also showed that Minghui 63(*Xa21*) and its F_1 hybrid, Shanyou 63(*Xa21*) produced the same level and spectrum of resistance as IRBB21, the donor parent of the *Xa21* gene. We further showed that Minghui 63(*Xa21*) and Shanyou 63(*Xa21*) have identical performance to the original Minghui 63 and Shanyou 63 under nondiseased conditions, but have significantly better performance than the original Minghui 63 and Shanyou 63 under heavily diseased conditions.

There are several points that need discussion regarding the efficiency of the MAS procedures in the recurrent backcross scheme. The first point concerns the selection for recombination between the targeted gene locus and the flanking markers. There are two options for obtaining an individual with recombination on both sides of the targeted gene. The first option is selection for simultaneous recombination on both sides, which will result in the desired recombinant in one generation. The alternative is a tandem selection, i.e., selection for recombination on one side in the first generation and selection for recombination on the other side in the next generation. Although the first option would save one generation of backcrossing, it is much more costly than the second option. For example, assuming a distance of 1 cM on both sides, simultaneous recombination on both sides would occur at a frequency of 0.01%. Thus, one would have to screen 10 000 positive individuals containing the gene (representing only 50% of the plants in a backcross population) to obtain one double recombinant. This is obviously prohibitive for the molecular marker assay as well as for the hand emasculation involved in making the cross. Additionally, there are possible complications of interference in obtaining a double cross over. In contrast, also assuming a distance of 1 cM on both sides, a recombinant event between the targeted gene and a flanking marker on either of the two sides would occur at a frequency of 2%. Thus, only 50 positive individuals would be needed in the first generation of backcrossing to expect a recombinant event between the targeted gene and one of the flanking markers, and 100 positive individuals would be needed to obtain a recombinant on the other side of the gene in the second generation of backcrossing. Thus, the second option obviously costs much less in labor and resources than the first option.

The second point is related to the time in which background selection should be practiced. At a single locus, the expected frequencies for individuals to be homozygous for the genotype of the recurrent parent would be 0.5, 0.75, and 0.875, in BC_1F_1, BC_2F_1, and BC_3F_1, respectively. These can also be viewed as the expected proportions of loci for individuals to be homozygous for the recurrent parent genotypes. The variances of such proportions in these generations would be $0.5(1 - 0.5)/n$, $0.75(1 - 0.75)/n$, and $0.875(1 - 0.875)/n$, respectively, where n is the number of independent recombinational units in the genome. It is not known how many map units are equivalent to an independent recombinational unit in the rice genome. But it is clear that the variance in BC_1F_1 is much larger than those in subsequent generations, indicating a wider frequency distribution in the BC_1F_1 than the later generations. Also, as discussed in the previous paragraph, the desired individual with recombination between the targeted gene locus and either one of the flanking markers is expected to occur at a much higher frequency in BC_1F_1 than BC_2F_1, which means that it is feasible to practice background selection in the BC_1F_1 generation. Thus, in addition to the background selection in the BC_3F_1 generation, adding one round of background selection in BC_1F_1 to the MAS scheme may greatly increase the efficiency of the program.

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