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Pyramiding *Xa23* and *Rxo1* for resistance to two bacterial diseases into an elite indica rice variety using molecular approaches

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Abstract Rice bacterial leaf blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* and bacterial leaf streak (BLS) caused by *X. oryzae* pv. *oryzicola* (*Xoc*) are two important diseases of rice that often outbreak simultaneously and constrain rice production in much of Asia and parts of Africa. Developing resistant cultivars has been the most effective approach to control BB, however, most single resistance genes have limited value in breeding programs because of their narrow-spectrum of resistance to the races of the pathogen. By contrast, there is little progress in breeding varieties resistant to *Xoc* since BLS resistance in rice was a quantitative trait and so far only a few quantitative resistance loci have been identified. We reported here the development of a high yield elite line, Lu-You-Zhan highly resistant to both BB and BLS by pyramiding *Xa23* with a wide-spectrum resistance to BB derived from wild rice and a non-host maize resistance gene, *Rxo1*, using both marker assisted selection (MAS) and genetic engineering. Our study has provided strong evidence that non-host R genes could be a valuable source of resistance in combating those plant diseases where no single R gene controlling high level of resistance exists and demonstrated that MAS combined with transgenic technologies are an effective strategy to achieve high level of resistance against multiple plant diseases.

Keywords Rice · Non-host resistant · Genetic engineering · Marker-assisted selection · Bacterial leaf streak · Bacterial leaf blight

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Introduction

Rice bacterial leaf blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most widely distributed and devastating rice diseases worldwide (David et al. 2006). Bacterial leaf streak (BLS) caused by *X. oryzae* pv. *oryzicola* (*Xoc*) is another serious disease of emerging importance that constrains rice production in certain rice growing regions in China and South/Southeast Asia (Tang et al. 2000).

The epidemic of both BB and BLS of rice requires similar environmental conditions and thus the two diseases often occur and outbreak simultaneously in many rice fields of the Central and South China, resulting frequently in heavy yield losses of rice (Zeng and Lin 2003).

For controlling the diseases, developing resistant cultivars is the most economic and effective method (Ogawa 1993). To date, at least 30 resistance (R) genes to BB have been registered (David et al. 2006). Of these, *Xa3* and *Xa4* have been widely used in breeding programs worldwide and played an important role in controlling the disease in Asia since 1970 s. Currently, almost all commercial indica hybrid and inbred cultivars in China are known to have *Xa4*, whereas *Xa3* is common in japonica varieties (Zhang 1991). This narrow genetic basis for resistance to BB in Chinese rice cultivars has imposed strong selection on the pathogen population, resulting in a dramatic increase in frequency of pathotype V that is virulent to both *Xa4* and *Xa3* during the past decade in China (Zeng et al. 2002). A different R gene, *Xa21* from a wild rice species, *O. longistaminata* has a broad resistance-spectrum to BB races (Khush et al. 1990). This R gene was cloned (Song et al. 1995) and thus been widely used in rice breeding programs by conventional breeding approach, marker-assisted selection (MAS) and genetic transformation (Tu et al. 1998; Zhai et al. 2001). Unfortunately, *Xa21* was again broken down recently by new virulent races in Philippines, Korea, India and China (Lee et al. 1999; Marella et al. 2001; Zeng et al. 2002). In searching for new R genes conferring stable resistance to BB, *Xa23*, a single completely dominant R gene identified from a wild rice species of *O. rufipogon* emerged as the most promising one, as it has a broad spectrum of resistance to all tested Chinese BB races during all developmental stages of rice (Zhang et al. 1998, 2001).

In contrast, no single R genes controlling high level of resistance to BLS have been identified in the primary gene pool of rice (Zhao et al. 2004), even though both BB and BLS are caused by two highly related bacterial species of *Xanthomonas oryzae* (Vauterin et al. 1995). BLS resistance in rice was reportedly a quantitative trait (Xu et al. 1997; Tang et al. 2000). Although the recent identification of quantitative resistance loci (QRLs) has provided new opportunities for improving BLS resistance through MAS (Tang et al. 2000; Zheng et al. 2005; Chen et al. 2006), no MAS efforts have been taken to transfer any identified QRLs to improve BLS resistance in breeding programs because of the complicated nature of QRLs (Li 2001; Li et al. 2006). Recently, a single maize dominant R gene, *Rxo1,* is reportedly to confer a high level of non-host resistance to rice BLS caused by *Xoc* (Zhao et al. 2004, 2005), providing an alternative strategy for breeding resistance to BLS.

Here we report an effort in developing elite rice variety resistant to both BB and BLS using combined approaches of phenotypic selection, MAS of *Xa23* from wild rice and *Agrobacterium tumefaciens* mediated transformation of the maize R gene, *Rxo1*, for BLS resistance.

Materials and methods

Plant materials

A rice line, CBB23-2 carrying *Xa23*, was used as the donor for resistance to BB and a commercial indica variety, Lu-You-Zhan (LYZ) was used as the recipient. LYZ is a high yielding cultivar in South China with excellent grain quality, but is highly susceptible to both BB and BLS. CBB23-2 was a BC_4F_5 progeny derived from a cross between an *O. rufipogon* accession (RBB16, the *Xa23* donor) and a recipient indica line, Jingang 30 which is highly susceptible to virtually all Chinese *Xoo* races (Zhang et al. 2001).

Maker-assisted transfer of the *Xa23* gene into rice variety LYZ

CBB23-2 was used as the male donor and crossed with LYZ to obtain the F_1 plants. The true F_1 plants were backcrossed to LYZ to obtain the BC_1F_1 plants. Resistant BC_1F_1 plants determined by MAS and artificial inoculation were further backcrossed to LYZ to produce the BC_2F_1 population. Consecutive backcrossing was carried out in the same way to produce BC_3F_1 and BC_4F_1 populations. The BC_4F_1 plants were then selfed twice to produce BC_4F_3 families. In each BC generation, resistant plants carrying *Xa23* were determined by MAS using an SSR marker, RM206 which is 1.9 cM from the *Xa23* locus (Pan et al. 2003; http://www.gramene.org), and by

artificial inoculation at the 4–5 leaf stage with the most virulent Chinese *Xoo* strain, GD1358. In the MAS process, the RM206 genotypes were used to infer the genotypes of BC progeny at the *Xa23* locus. Finally, one BC_4F_5 line carrying the homozygous *Xa23* gene and LYZ agronomic phenotype was identified and designated as LYZ-*Xa23*, which was used as the recipient for transformation of *Rxo1*.

Vector with *Rxo1* and rice transformation

The plasmid pCAMBIA1305-1, which contains the whole *Rxo1* gene in an approximately 9 kb *Eco*R I fragment, was kindly provided by Dr. SH Hulbert of Kansas State University, USA. In this study, the binary vector pMNDRBBin6 was used for producing selectable marker-free transgenic plants (Lu et al. 2001), a digested *Sca*I-*Rxo1*-*NgoM*IV fragment of *Rxo1* from pCAMBIA1305-1 was ligated into the *Xma*I-*Hpa* digested pMNDRBBin6 to obtain the recombinant plasmid pMNDRBBin6-*Rxo1*, which has the structure of RB1-*hph*-RB2-*Rxo1*-LB (Fig. 1) (Xu et al. 2008). pMNDRBBin6-*Rxo1* is a double right-border (DRB) vector carrying two copies of T-DNA right-border (RB) sequences flanking the selectable gene, hygromycin phosphotransfase (*hph*), followed by the *Rxo1* gene and one copy of the left border (LB)sequence. Two types of T-DNA inserts, one initiated from the first RB containing the selectable gene and *Rxo1* gene, and the other from the second RB containing only *Rxo1*, were produced and integrated into the genome. The vector pMNDRB-Bin6-*Rxo1* was introduced into an *A. tumefaciens* strain, EHA105 through electroporation and used in rice transformation. The LYZ-*Xa23* plants were used as the recipients of the *Rxo1* gene, as described above. Rice transformation was carried out according to the method described by Zhai et al. (2000).

Molecular analysis of transgenic plants

Two primer pairs were used for molecular analyses of transgenic plants. The first pair from the *Rxo1* gene sequence was R_1 (5′-ACTATCGGCGAGTACTTCT ACACAG-3′) and R_2 (5′ - GAGTTTAGCGAGAGC CTGACCTAT-3′) with a 1.45 kb amplified fragment. The second one designed for specific PCR amplification of *hph* was H_1 (5′- TAGGAGGGCGTGG ATATGC-3′) and H_2 (5′-TACACAGCCATCGGTC CAGA-3′) with a 1.1 kb amplified fragment. All PCR reactions were carried out in 25 μl mixtures containing 50 ng genomic DNA, 10 mmol/l Tris–HCl pH9.0, 50 mmol/l KCl, 1.8 mmol/l $MgCl_2$, 200 μmol/l dNTPs, 50 ng of each primer, and 1 unit of *Taq* DNA polymerase. PCR reactions were performed in a PTC-100 ^{M} Programmable Thermal Controller (MJ Research Inc.) using the following program: predetermination at 94°C for 5 min; then 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min for 35 cycles; followed by 10 min at 72°C. Electrophoresis of the PCR products were carried out on 1.0% agarose gels in 1 × TAE buffer.

In addition, 5–10 μg genomic DNA was extracted from each transgenic line, digested with *EcoR* I and electrophoresed on a 0.8% agarose gel. Southern hybridization was carried out with ECL^{TM} Direct Nucleic Acid Labeling and Detection System as described by the manufacturer, Amersham. The probes used were the 1.45 kb and 1.1 kb PCR fragments amplified by primer pairs R_1/R_2 and H_1/H_2, respectively for confirmation of the *Rxo1* gene and *hph* in the transgenic plants.

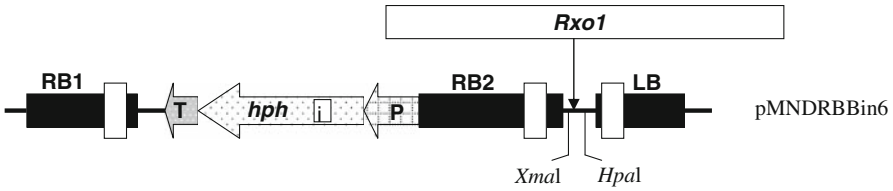

Fig. 1 Diagram of the plasmid of binary vector, pMNDRB-Bin6-*Rxo1* Double right-border (DRB) binary vector pMNDRBBin6 described in detail by Lu et al. (2001). RB1, the right border 1 of T-DNA; *hph*, hygromycin phosphotransfase gene; *N*, terminator of *nos* gene; *P*, CMV35S promoter; RB2, the right border 2 of T-DNA; *Rxo1*, the *Rxo1* gene; LB, the left border of T-DNA. The *Sca* I and *NgoM* IV fragment containing 8.5 kb *Rxo1* gene was excised from pCAM-BIA1305-1 and ligated into the site *Hpa*I and *Xma* I of pMNDRBBin6 to produce pMNDRBBin6-*Rxo1*

Screening of selectable marker-free transgenic plants with *Rxo1*

The independent transformants of T_0 generation were analyzed with primer pairs R_1/R_2 and H_1/H_2 and confirmed by artificial inoculation with *Xoc* strain GD4. T_1 seeds were obtained by selfing the highly resistant and positive plants. Segregating plants of T_1 generation were further analyzed by PCR and inoculation. Seeds of the plants with *Rxo1* but without *hph* were harvested. The selectable marker-free transgenic plants were identified from the homozygous resistant lines in T_2 generation by artificial inoculation and PCR, and confirmed by Southern hybridization with the probes of *Rxo1* and *hph*.

Artificial inoculation and resistance evaluation

The plants were grown in the screenhouse of the Institute of Crop Sciences (ICS), Chinese Academy of Agricultural Sciences, Beijing, China in the summer of 2003–2006. For evaluating BB resistance, 4–5 uppermost leaves of each plant were inoculated with *Xoo* strains at the seeding or tillering stage by the leaf-clipping method (Kauffman et al. 1973). The lesion length was measured on all inoculated leaves 2 weeks after inoculation. For evaluating BLS resistance, all transgenic plants were inoculated with *Xoc* strains GD4, FJ1, HB1, HN4 and HN5 collected from the rice fields in Gaungdong, Fujian, Hubei and Hainan provinces using a pin pricked method at the booting stage (Tang et al. 2000). The inoculated plants were scored for their resistances to BB and BLS according to the standard evaluation system of rice (IRRI 1996) 2 weeks after inoculation when lesions became obvious and stable in the susceptible check, LYZ.

To evaluate resistance of the resultant pyramids with *Xa23* and *Rxo1*, seven Chinese *Xoo* races (Fang et al. 1990), 10 Philippine *Xoo* races (Li et al. 2006), and five *Xoc* strains (GD4, FJ1, HB1, HN4 and HN5) were used to inoculate the pyramiding plants in the ICS screenhouse using the same methods described above.

Results

Inheritance of the *Xa23* gene in the F_1 and BC_1F_1 plants

Seventeen F_1 plants were obtained from the cross of LYZ × CBB23-2. Genotypic data at SSR marker RM206 indicated that all 17 plants had *Xa23*. The F_1

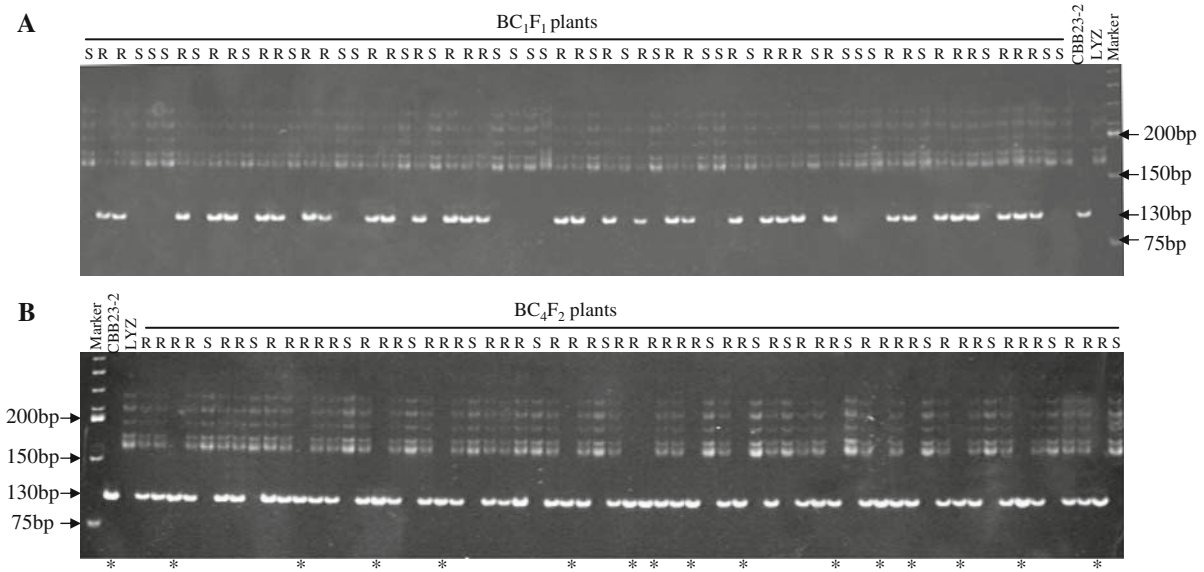

Fig. 2 Segregation of the *Xa23* mediated resistance to BB based on the marker genotypes at RM206 which is tightly linked to *Xa23* in BC1F1 (**a**) and BC4F2 (**b**) populations derived from the cross between LYZ (the recipient) and CBB23-2 (the donor). Letters *S* and *R* represent the susceptible and resistant reactions of plants to strain GD1358 of *X. oryzae* pv. *Oryzea*, respectively. The markers on the *right* (**a**) and *left* (**b**) were the 50 bp ladder (NEB Inc.). The arrow at the 130 bp is *Xa23*-specific DNA fragment. * denotes the homozygous plants with *Xa23*

plants were resistant to all seven Chinese *Xoo* races with an average lesion length of 0.4 ± 0.3 cm, whereas LYZ was susceptible to all tested strains with an average lesion length of 12.5 ± 1.9 cm. The *Xa23* donor, CBB 23-2, had an average lesion length of 0.4 ± 0.2 cm. This result indicated that *Xa23* is a completely dominant R gene.

Of the 156 BC_1F_1 plants, 75 plants were inferred to be heterozygous at the *Xa23* locus based on their genotypes at RM206 (Fig. 2a). Inoculation with GD1358 at the tillering stage indicated 74 out of 75 plants were resistant with an average lesion length of 0.6 ± 0.2 cm, indicating a genetic distance of 1.3 cM between RM206 and *Xa23* and a high accuracy of 98.7% in our MAS for *Xa23* using marker RM206.

Development of LYZ-*Xa23* through MAS

Five plants having *Xa23* and phenotypically similar to LYZ were selected from 156 BC_1F_1 plants based on MAS and inoculation confirmation to backcross to LYZ, resulting in a total of 356 BC_2F_1 progenies. Six resistant plants phenotypically similar to LYZ were selected from the BC_2F_1 progeny using the same procedure to further backcross with LYZ, producing 410 BC_3F_1 progeny. Then, seven plants with *Xa23* selected from the BC_3F_1 progeny using the same methods were continuously backcrossed to LYZ, resulting in 475 BC_4F_1 progeny. Selfed BC_4F_2 seeds were harvested from five BC_4F_1 plants that had *Xa23* and LYZ phenotype. Genotyping of 450 BC_4F_2 progeny from the five selected BC_4F_1 plants with RM206 revealed all three genotypes at RM206 (Fig. 2b), including 26 plants homozygous for the resistant allele and similar to LYZ in phenotype. Resistance of the 26 resultant BC_4F_3 lines was further confirmed by inoculation. From these lines, one line phenotypically identical to the control (Table 1), LYZ was selected and designated as LYZ-*Xa23*.

Production of transgenic plants with the *Rxo1* gene

The selected LYZ-*Xa23* line was transformed with the binary vector, pMNDRBBin6-*Rxo1*, and 21 T_0 transformants were obtained, 11 of which were true transgenic plants based on the presence of the expected 1.45 kb *Rxo1* fragment and 1.1 kb *hph* fragment in these plants when amplified by primer pairs R_1/R_2 and H_1/H_2 (Fig. 3). Six of the T_0 transformants only produced the 1.1 kb *hph* fragment. The remaining four transformants were false positives since they produced neither the *Rxo1* fragment and nor the *hph* fragment. The 11 true transgenic plants exhibited a hypersensitivity-like reaction to the *Xoc* strain, GD4 with restricted necrotic lesions in the pricking areas 2 days after inoculation, whereas the check LYZ showed rapidly spreading water-soaked lesions. However, these transgenic lines differed slightly in their levels of resistance with mean lesion lengths ranging from 0.31 to 0.85 cm 2 weeks after inoculation.

Generation of selectable market-free transgenic lines

T_1 plants derived from seven of the resistant T_0 lines were segregating in resistance. Molecular analyses of these plants with H_1/H_2 identified a total of eight plants which had the 1.45 kb *Rxo1* fragment but no *hph* fragment, suggesting they were selectable marker-free. These included four plants from line L2, three plants from line L7 and only one plant from line L15, from which five plants with the homozygous *Rxo1* gene and LYZ –*Xa23* phenotype were selected.

Southern analysis confirmed the results from the PCR analyses that there was the DNA band corresponding to the *Rxo1* gene (Fig. 4), but no band for *hph* in the five selected T_2 plants. This result indicated that the *Rxo1* gene had been stably inherited

Table 1 Comparison of agronomic traits of LYZ-*Xa23* line and LYZ

Line name	Number of panicles per plant	Growth duration (days)	Plant height (cm)	Panicle length (cm)	Grains per panicle	Setting rate (%)	1,000-grain weight (g)	Yield per plant (g)
LYZ (control)	11.1	128	106.2	22.1	168.6	76.1	19.8	28.2
LYZ-*Xa23*	11.8	129	106.6	22.8	166.2	77.1	20.5	30.9

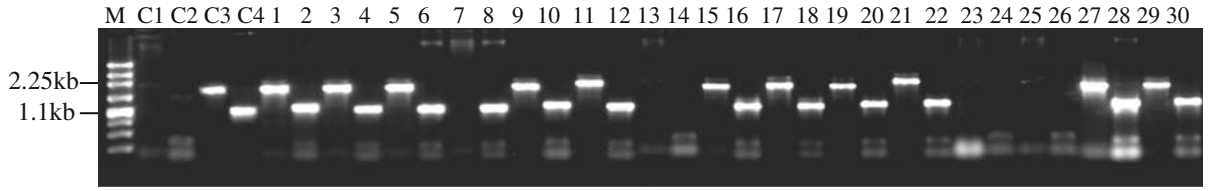

Fig. 3 PCR analysis of the transgenic T_0 plants. *M* represents 250 bp molecular weight marker (Takara Inc.); C1 and C2 represent the negative control of LYZ amplified by primer pairs R_1/R_2 and H_1/H_2, respectively. C3 and C4 represent the positive control of pMNDRBBin6-*Rxo1* amplified by R_1/R_2 and H_1/H_2, respectively. Codes 1–30 represent the transgenic lines. The neighboring odd and even codes were the same plants; the bands of odd codes (the 1.45 kb fragment) and even codes (the 1.1 kb fragment) were amplified by R_1/R_2 and H_1/H_2, respectively

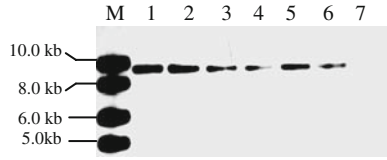

Fig. 4 Southern blot analysis of the T_2 transgenic plants (*lanes* 2–6) with the R_1/R_2 amplified 1.45 kb PCR fragment of *Rxo1* from pMNDRBBin6-*Rxo1* as the probe. *M* represents 1 kb molecular weight marker (NEB Inc.); *Lanes* 1 and 7 represent the digested vector pMNDRBBin6-*Rxo1* as the positive control and LYZ as the non-transformed control, respectively

and the vector backbone sequence was removed in these plants through recombination. The presence of *Xa23* in these transgenic lines was further confirmed with RM206. From these, one of the homozygous plants was designated as LYZ-*Xa23-Rxo1*.

Assessment of the pyramid for resistance to *Xoo* and *Xoc*

Table 2 shows the mean lesion lengths of LYZ-*Xa23-Rxo1*, LYZ-*Xa23* and the control LYZ caused by 10 Philippine *Xoo* races, seven Chinese *Xoo* races and five Chinese *Xoc* strains. LYZ was susceptible to all 17 *Xoo* races and exhibited rapidly spreading water-soaked lesions 24 h after inoculation with an average lesion length reached 8.1 ± 1.7 cm. The LYZ-*Xa23-Rxo1* and LYZ-*Xa23* lines both showed a high level of resistance to all *Xoo* races (including the most virulent Chinese race, GD1358) with virtually the same lesion lengths < 1.0 cm, indicating that the presence of *Rxo1* didn't affect the expression of *Xa23* in the LYZ-*Xa23-Rxo1* line (Fig. 5a). LYZ-*Xa23-Rxo1* also showed a high level of resistance to the highly virulent Philippine race 6, PXO99 at the 4–5 leaf stage (data not shown).

When inoculated with the five *Xoc* strains, LYZ-*Xa23-Rxo1* rapidly showed the hypersensitive reaction, and the lesion length of LYZ-*Xa23-Rxo1* was 0.27 cm 2 weeks after inoculation (Fig. 5b; Table 2), suggesting that the growth of *Xoc* in the pyramid was significantly inhibited.

Discussion

To date, most of the 30 reported R genes to BLB have not been used in breeding programs due either to their lower level of resistance or to their narrow spectrum of resistance (David et al. 2006). In this regard, single R genes with wide spectrum of resistance are of choice because of their effectiveness against more *Xoo* races and the easiness in manipulating target genes in breeding programs. Derived from wild rice *O. rufipogen*, *Xa23* has the broadest spectrum of resistance among all BB R genes identified and confers high level of resistance at all growth stages to the all tested strains, including 10 Philippine races, 7 Chinese pathotypes and 8 isolates from Korea (Zhang et al. 1998, 2001). As demonstrated in this study, the simple inheritance of *Xa23* as a single dominant R gene and availability of its closely linked markers make it easy to use *Xa23* in breeding programs by either phenotypic selection or MAS. We realize that *Xa23* will almost certainly be overcome by new virulent *Xoo* races sometime in future as long as it becomes widely used in breeding programs according to our knowledge and past experiences (Mew et al. 1992; Adhikari et al. 1995; McDonald and Linde 2002). Fortunately, this can be prevented by combing *Xa23* with other R genes such as *Xa4*, *xa5*, *xa13* and *Xa21* to achieve high level and durable resistance to *Xoo* before it is broken down.

Table 2 Resistance of rice lines LYZ-*Xa23*, LYZ-*Xa23-Rxo1*, and LYZ (the susceptible check) to *X. oryzae* pv. *oryzea* at the tillering stage and *X. oryzae* pv. *oryzicola* at the booting stage

	Strains	Races/Pathotypes	Origin	Lesion length (cm)		
				LYZ-*Xa23-Rxo1*	LYZ-*Xa23*	LYZ
BB	PXO61	Race1	Philippines	0.41 ± 0.14	0.44 ± 0.15	9.76 ± 1.20
	PXO86	Race2	Philippines	0.36 ± 0.12	0.38 ± 0.12	8.79 ± 0.65
	PXO340	Race3	Philippines	0.39 ± 0.12	0.32 ± 0.13	7.07 ± 2.37
	PXO71	Race4	Philippines	0.34 ± 0.14	0.41 ± 0.12	8.97 ± 3.18
	PXO112	Race5	Philippines	0.38 ± 0.16	0.41 ± 0.14	9.81 ± 2.85
	PXO99	Race6	Philippines	0.71 ± 0.13	0.87 ± 0.21	12.22 ± 0.74
	PXO145	Race7	Philippines	0.31 ± 0.20	0.32 ± 0.14	5.41 ± 1.74
	PXO280	Race8	Philippines	0.14 ± 0.05	0.16 ± 0.05	5.40 ± 1.30
	PXO339	Race9	Philippines	0.37 ± 0.17	0.22 ± 0.12	8.00 ± 1.72
	PXO341	Race10	Philippines	0.42 ± 0.16	0.49 ± 0.11	9.22 ± 2.70
	JS97-2	Pathotype I	China	0.36 ± 0.17	0.38 ± 0.22	8.61 ± 1.33
	KS6-6	Pathotype II	China	0.37 ± 0.21	0.31 ± 0.21	6.33 ± 1.84
	JS158-2	Pathotype III	China	0.38 ± 0.22	0.40 ± 0.13	8.07 ± 1.43
	Z173	Pathotype IV	China	0.32 ± 0.16	0.28 ± 0.08	7.75 ± 2.46
	GD1358	Pathotype V	China	0.82 ± 0.17	0.76 ± 0.24	11.14 ± 1.49
	OS198	Pathotype VI	China	0.30 ± 0.13	0.52 ± 0.22	6.25 ± 1.68
	JS49-6	PathotypeVII	China	0.42 ± 0.22	0.28 ± 0.12	5.03 ± 0.71
	Average			0.40 ± 0.16	0.41 ± 0.15	8.06 ± 1.73
BLS	GD4		China	0.32 ± 0.06	-	3.16 ± 0.52
	FJ1		China	0.28 ± 0.03	-	1.97 ± 0.31
	HB1		China	0.30 ± 0.04	-	1.73 ± 0.08
	HN4		China	0.25 ± 0.03	-	2.05 ± 0.89
	HN5		China	0.19 ± 0.02	-	1.68 ± 0.15
	Average			0.27 ± 0.04		1.91 ± 0.33

Non-host disease resistance refers to the phenomenon that most plant species are typically resistant to the pathogens of other plant species. This nature of non-host resistance implies that it is of broad spectrum and durable as compared with race-specific R genes of host plants (Heath 1987). As an old phenomenon, non-host resistance has not been used in any breeding programs due to it's speculated multiple protective mechanisms and the existence of reproductive barriers between different plant species (Thorsten and Volker 2005). Non-host resistance is typically thought to be controlled by multiple genes with general (non-specific) effects on microbial pathogens (Heath 2000). There is, however, growing evidence that single loci can contribute significant effects towards non-host resistance, and some genetic defects that affect active defense processes, like HR, also affect host's responses to non-host pathogens (Peart et al. 2002; Kang et al. 2003). Thus, there have been increasing interests in non-host resistance and its potential use in heterologous plant species by genetic engineering. The maize *Rxo1* gene is able to direct active defense responses that culminate in a rapid HR in a japonica rice (Zhao et al. 2004). Here, we transformed an elite indica rice variety LYZ-*Xa23* with *Rxo1*, further demonstrating that *Rxo1* could express stably and produce HR in a heterologous indica background. Our results further indicated that *Xa23* and *Rxo1* control resistance to BLB and BLS, respectively, and they appeared to function independently.

Achieving a high level of resistance to BLS in the LYZ-*Xa23-Rxo1* line in this study was consistent with previously reported results (Zhao et al. 2005) and

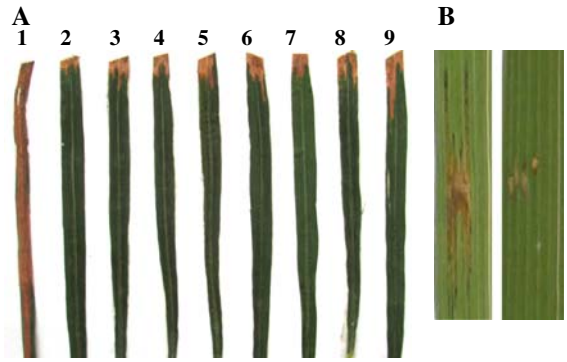

Fig. 5 Resistant reactions of pyramid line LYZ-*Xa23-Rxo1* to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *X. oryzae* pv. *oryzicola* (*Xoc*). **a** Resistant reactions of LYZ-*Xa23-Rxo1* plants to *Xoo*. Leaves 1 and 2 represent the reactions of susceptible check LYZ and resistant check CBB23-2 to Chinese pathotype V, GD1358; Leaves 3–8 represent the reactions of the LYZ-*Xa23-Rxo1* plants to 7 Chinese *Xoo* pathotypes, JS97-2, KS6-6, JS158-2, Z173, GD1358, OS198 and JS49-6, respectively. **b** Resistant reactions of LYZ-*Xa23-Rxo1* and LYZ (the susceptible check) to *Xoc* strain GD4 2 weeks after inoculation. *Left* and *right* are the leaves of the non-transgenic LYZ and LYZ-*Xa23-Rxo1* plant, respectively

demonstrated that non-host R genes combined with transgenic technologies are an effective strategy to achieve enhanced resistance against plant diseases in which no high level of resistance controlled by single R genes exists in the host plant species. The strong expression of *Rxo1* mediated HR to BLS in both japonica (Zhao et al. 2005) and indica genetic backgrounds demonstrated in this study foresees a wide application of *Rxo1* to combat BLS in future rice breeding programs. Although *Rxo1* is a single NBS-LRR type of R gene from maize capable of inducing non-host resistance to rice BLS (Zhao et al. 2005), the nature of non-host resistance itself (Heath 1987; Thorsten and Volker 2005) and the lesser degree of differentiation in virulence of the *Xoc* populations imply that the BLS resistance coffered by *Rxo1* might be more durable. However, it would also be very interesting to see how the *Xoc* populations evolve in responses to *Rxo1* after it is widely used in rice breeding programs. Thus, as the major advancement to be achieved in the functional genomic research of major cereals and other model plants (http://www.sanger.ac.uk; http://www.ifti.org; http://www.seqnet.dl.ac.uk) in near future, more and more non-host R genes are expected to be discovered and cloned, providing tremendous opportunities in combating a wide range of plant diseases in agriculture.

Developing superior crop varieties with high level and durable resistance to multiple diseases has been a major challenge to plant breeders and pathologists. In this study, we developed a new high yielding LYZ line pyramided with *Xa23* and *Rxo1*, which represent firstly successful case in achieving this goal using both host and non-host R genes by MAS and genetic engineering. The resultant LYZ-*Xa23-Rxo1* line showed a high level of resistance to both BB and BLS, which can be released for commercial use in the epidemic areas of BLB and BLS in Central and South China, or to be used as donor in rice breeding programs for combating BB and BLS within China and elsewhere. Our study has provided additional evidence that non-host R genes could be a valuable source of resistance in combating those plant diseases where no single-R-gene controlled HR exists.

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