

SHORT COMMUNICATION

ASSESSMENT OF A FUNCTIONAL MARKER FOR BACTERIAL BLIGHT RESISTANCE GENE LOCUS *Xa 27* IN RICEP. Yu¹, X.M. Wang², X.P. Yuan¹, C.H. Wang¹, Q. Xu¹, Y. Feng¹, H.Y. Yu¹, Y.P. Wang¹ and X.H. Wei¹¹State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou, China²Crop Research Institute, Shandong Academy of Agricultural Sciences, Jinan, China

SUMMARY

The bacterial blight gene locus *Xa 27* in rice has two striking differences in the presumed promoter-raised resistant/susceptible allele expression. We used a functional marker targeting the 25 deletion at 18 bp upstream of the putative TATA box and assayed the allelic status at *Xa 27* in 572 rice genotypes. The alleles evaluation obtained from 191 cultivars tested may contribute to little information of the resistance specificity associated with allele diversity. The allele frequency associated with complete resistance in the *indica* was different from that associated with complete resistance in the *japonica*.

Key words: rice, bacterial blight, resistance gene *Xa 27*, allele variation

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is one of the serious diseases of rice prevalent throughout Asia (Reddy, 1989; Gnanamanickam *et al.*, 1999). Chemical control is not practical or effective (Sidhu *et al.*, 1978), hence the use of resistant varieties is the most economical, feasible, and environmentally sound mean of controlling the disease (Khush *et al.*, 1989; Ogawa 1993). The genetic basis of host resistance to BB has been studied in great detail. More than 30 genes have been reported, some of them were mapped by various scientists on the genetic map of rice. Of these, *Xa 27* was transferred from the tetraploid wild rice *Oryza minuta* to the cultivated rice species, *Oryza sativa* (Amante-Bordeos *et al.*, 1992). Gu *et al.* (2004) initiated fine-scale genetic mapping of *Xa 27* locus. *Xa 27(t)* was finally located within a genetic interval of 0.052 cM, flanked by markers M964 and M1197 on chromosome 6.

As a prelude to cloning *Xa27(t)*, *Xa 27* was isolated from rice cv. IRBB27 by map-based cloning (Gu *et al.*, 2005). The allele from susceptible cv. IR24 was also characterized

and shares a near identical DNA sequence with *Xa 27* from the resistant cv. IRBB27. Apart from this, two striking differences in the presumed promoter were detected between IR24 and IRBB27, resulting in the presence of a 10 bp sequence at the -1.4 kb position and a 25 bp duplication at 18 bp upstream of the putative TATA box in the susceptible allele (Fig. 1). These differences raised the possibility that the two alleles might differ in their expression. The objectives of the present study were: (i) identify allelic variation carrying the 25 bp deletion within the gene *Xa27* promoter TATA box site in a wide collection of rice germplasm including Chinese rice varieties (*O. sativa*), African cultivated rice (*O. glaberrima*) and wild relatives; (ii) investigate the effects of various alleles for resistance reaction and identify potential genetic resources for breeding rice for resistance.

The previously developed primer pair *Xa27F* (5'-TAGT-GTCTAAATACAGGGACT-3'), and *Xa27R* (5'-GAG-TACTTTGCTCTGATGCTC-3') targeting the sequences flanking the 25 bp deletion specific for the promoter TATA box site of *Xa 27* allele was used (Xia *et al.*, 2009). A total of 572 accessions including 495 cultivars, 12 African cultivated rice (*O. glaberrima*), and 65 AA-genome wild rice accessions (*O. rufipogon*, *O. nivara*, *O. barthii*, *O. glumaepatula*, *O. longistaminata*, and *O. meridionalis*) were analyzed for assessing the allelic status at promoter of *Xa 27*. Phenotyping for resistance was performed as described by Amante-Bordeos *et al.* (1992). Genomic DNA was isolated from leaf young leaves by CTAB extraction (Murray and Thompson, 1980). PCR reactions were set up as follows: 10× PCR buffer, 0.2 mM dNTP, 1.0 mM of each primer, 0.5 U of *Taq* DNA polymerase (DINGGUO, China), and sterile water to final volume of 10 ml. PCR was performed using an Applied Biosystems 2720 Thermal Cycler with the following PCR conditions: 94°C 5 min, (94°C 45 sec, 55°C 45 sec, 72°C 1 min)×30 cycles, 72°C 8 min. PCR products were analyzed by electrophoresis on a 6% polyacrylamide gels and detected by silver staining (Ji *et al.*, 2007).

Five alleles were detected when the primer pair *Xa27* was used for amplification (Table 1). The PCR product 171 bp in size have the 25 bp at the promoter TATA box site, scored as type I allele (i.e., IR24-allele). However, Type I

Table 1. Alleles and their frequencies at marker Xa27 in various populations sampled.

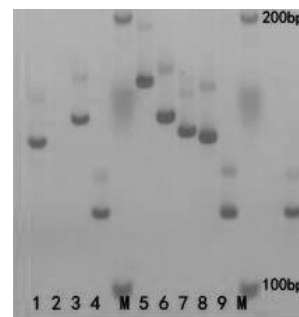
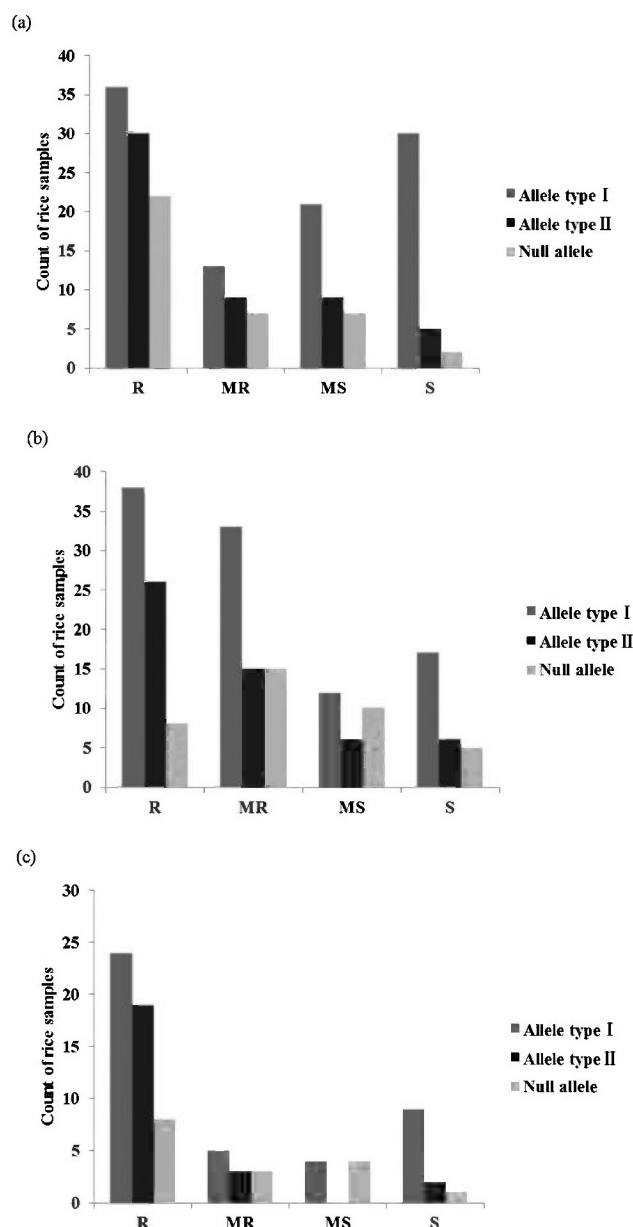
Marker	Allele		Allelic frequency		
	Name	Size (bp)	All accessions	<i>O. glaberrima</i>	wild rice
Xa27	Type I	171	39.69%	-	1.54%
	Type II	146	28.67%	-	49.23%
	Type III	149	0.17%	-	-
	Type IV	160	1.05%	-	9.23%
	Type V	120	0.35%	-	3.08%
	Null allele		30.07%	100.00%	36.92%

IR24 GAAGTTAGAACTCTACCAACGGATAGTTAATTGGAATTTGGAT
IRBB27 GAAGTTAGAACTCTACCAA-----ATTGGAATTTGGAT
IR24 ACTCTAAATTAGTGTCTAAATACAGGGACTAAAGTAAAGTCCCTG
IRBB27 ACTCTAAATTAGTGTCTAAATACAGGGACTAAAGTAAAGTCACTG
IR24 GATCCAAACGCCACCTAAGGTTTTCTTGTGTACTTGTGAATTGTG
IRBB27 GATCCAAACACCACCTAAGGTTTTCTTGTGTACTTGTGAATTGTG
IR24 GTTCTGTGTACTTGTGAATTGTGGTTGACTACGACTACGAGTG
IRBB27 GTT-----GACTACGACTACTAGTG
IR24 CTATAAATAAGAAG-----GACCAATAGAGAGCATCAGAGCAAAGT
IRBB27 CTATAAATAAGAAGAGACCCATAGAGAGCATCAGAGCAAAGT
IR24 ACTCCTAAAAGACGCCACACACTGAGACACCCAAGAAGCTG
IRBB27 ACTCCTAAAAGACGCCACACACTGAGACACCCAAGAAGCTG
IR24 CCTCCAATGGCGGATTGGGCGATGCACCACTACCTCCTACTAGCC
IRBB27 CCTCCAATGGCGGATTGGGCGATGCACCACTACCTCCTACTAGCC

Fig. 1. Genomic organization and sequence alignment of the polymorphic promoter regions (dotted line) of *Xa 27* alleles. The primer pair Xa27 is underlined. TATA boxes (bold) and start codons (italics) are indicated.

allele was observed with a frequency of 39.69% in all accessions, which included 226 cultivars and one wild rice. Interestingly, the PCR products of the susceptible parent CO39 did not carry the 171 bp band (Fig. 2). The products of 164 accessions were a smaller fragment, hereafter referred as type II allele (approximate 146 bp). Notably, the 149 bp band pattern (Type III allele) was unique to IRBB27. Five *O. rufipogon* and one *O. barthii* with 160 bp band were observed and taken as type IV, two *O. barthii* had the type V allele fragment 120 bp in size. The allele in cv. Nipponbare diverged considerably (64% identity) from the gene of IRBB27 by sequence analysis. This observation remains true since we were not able to amplify from cv. Nipponbare genomic DNA. Our experiment data suggests that 172 genome organizations (including 136 cultivars, 12 *O. glaberrima* and 24 wild relatives) might be similar to cv. Nipponbare.

Allele diversity and phenotype reaction were assessed in 191 accessions of *Xoo* strains (PXO86, PXO112). In this work, each allele was not found in a single resistance reaction group, rather all allele types were found crosswise in resistant/susceptible group, such as the resistant accessions had the same allele as susceptible accessions (Fig. 3). Analysis of allele diversity for *Xa 27* indicates that the *xa27* resistance phenotype either derives from multiple origins or is

**Fig. 2.** The marker Xa27 can clearly distinguish all five allelic status at the promoter TATA box site of *Xa 27*. Lanes 1-9: YP14 (*O. nivara*), Nipponbare, YP33 (*O. barthii*), YP34 (*O. barthii*), IR24, YP21 (*O. rufipogon*), IRBB27, CO39, YP36 (*O. barthii*).**Fig. 3.** Disease reactions of selected rice samples to PXO112 (a), PXO86 (b), both stains (c) and their allelic status at the promoter TATA box site by the marker Xa27. R: resistant, MR: middle-resistant, MS: middle-susceptible, S: susceptible.

genetically heterogeneous. In addition, *Xoo* strains contain multiple avirulence proteins, which could interact with susceptibility alleles at other loci, so the susceptibility could be caused by other loci. However, allele frequency associated with complete resistance in var. *indica* was different from that associated with complete resistance in var. *japonica*.

In conclusion, the marker used in the present work was mainly able to distinguish the “IR24-type” *Xa* 27 alleles from the homologues found in IRBB27, cv. Nipponbare, and a larger set of rice accessions. At this time, little information is available on the distribution of alleles in resistance reaction. This result provides a valuable experience for routine identification of the allelic status at resistance gene locus in rice. Importantly, we found that the allele frequency associated with complete resistance in var. *indica* was different from that of var. *japonica*. However, since the *Xa* 27 function may extend beyond a single base, studies will require sequencing of larger samples so as to capture more useful mutation events and abundant haplotypes on some positions.

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