

SHORT COMMUNICATION

SEQUENCE VARIATIONS AND HAPLOTYPES
OF THE BACTERIAL BLIGHT RESISTANCE GENE *xa13* IN RICEP. Yu^{†1}, X.M. Wang^{†2}, 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 310006, China²Crop Research Institute, Shandong Academy of Agricultural Sciences, Jinan 250100, China[†] These authors contributed equally to this work

SUMMARY

The sequence variation diversity in the rice bacterial blight (BB) resistance locus *xa13* was evaluated in 217 rice cultivars in this study. On the basis of 51 polymorphic sites in the promoter region, eighteen distinct haplotype groups were detected, with a haplotype diversity of 0.642. A 252 bp insertion in the -69 bp to -86 bp region of the promoter is useful for association with BB resistance. Six haplotype groups were developed from the five SNPs in the *xa13* gene coding region. Alignment of the deduced amino acid sequences supported the existence of two XA13 protein haplotypes. The haplotypes of *xa13* did not segregate in *japonica* and *indica*, the natural occurring alleles of *xa13* had no independent origins in the two subspecies.

Key words: Rice, bacterial blight, *xa13*, single nucleotide polymorphism (SNP), haplotype

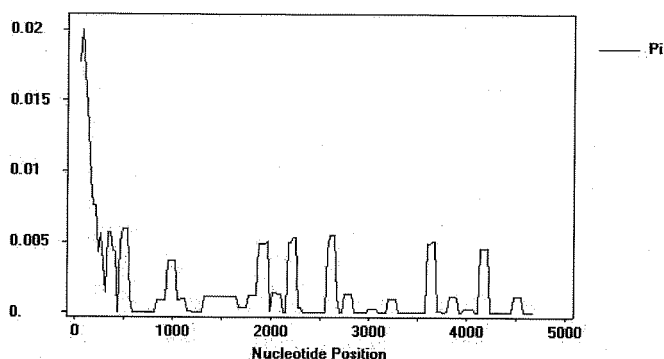
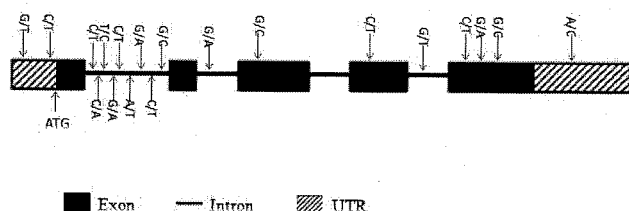
Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is one of the most devastating bacterial diseases and a major constraint to rice production worldwide (Gnanamanickam *et al.*, 1999). At present, a number of resistance genes have been identified, and some of them have been used in rice breeding for BB resistance (Yoshimura *et al.*, 1996; Blair *et al.*, 2003; Luo *et al.*, 2012). Rice plants can generate new resistance gene alleles through gene diversification, which allow them to adapt rapidly to a broad spectrum of novel pathogen variants. For this reason, evaluation of sequence variation of resistance genes among subspecies or accessions is required for exploration of new and favorable allelic variations.

Previous study has shown that the Xoo resistance of rice plants carrying the recessive *xa13* gene is due to a

mutation of a *cis*-acting element, the UPTPhXol box in the *xa13* promoter (Chu *et al.*, 2006; Yuan *et al.*, 2009). In the present study, forward and reverse primers were designed to amplify different overlapping segments covering the entire open reading frame (ORF) of *xa13* as well as approximately 1,800 bp upstream of the start codon and approximately 600 bp downstream of the translation stop codon (Table 1). The PCR-derived amplification products were sequenced by the Sanger dideoxy method on an Applied Biosystems 3730 DNA sequencer. The individual raw DNA sequences were assembled into contigs using a combination of Chromas and ContigExpress software. Introns and exons were predicted in all selected cultivars on the basis of sequence conservation of intron/exon boundaries in the corresponding IRBB13 sequence (GenBank accession No. DQ421394). To analyze variation, nucleotide diversity was estimated in DnaSP version 5.1 (Librado and Rozas, 2009) after applying a Jukes-Cantor (Jukes and Cantor, 1969) correction to each pairwise comparison. A sliding window analysis was performed to reveal changes in the level of variation along the *xa13* gene. Notably, the nucleotide diversity of the promoter region was higher than that of either the *xa13* ORF or the 3' region (Fig. 1). We sequenced an approximately 1,800 bp region upstream of the 5' start codon in 217 rice varieties (Supplemental Table 1) and compared these sequences with the IR24 promoter sequence (DQ421396). On the basis of 51 polymorphic sites in the aligned sequence data set, the 217 varieties could be classified into 18 distinct haplotype groups, with a calculated haplotype diversity of 0.642. Eleven of these haplotypes were rare and represented by only one variety each, namely, Basmati 370, Kalimakri77-5, Nannong219, NGA YA PAUK, Pankhari 203, JAVA14, Aus123, BG35-2, Aus242, Gui630, and 32xuan5. The two most common haplotypes were found in 28.11 and 52.53% of the studied varieties. Sequence analysis revealed that a 252 bp insertion in the -69 bp to -86 bp region of the *xa13* promoter was unique to three varieties with high BB resistance: IRBB55, Long Grain (35023), and IR 77542-560-1-1-1-2. This site is useful for association studies concerning *xa13* allelic polymorphism and BB resistance.

Table 1. Primers used for amplification of the *xa13* locus.

Locus	Forward	Reverse
Fragment 1	TCGGAACCTCCCAATACGATAT	CTCGCTACTGATGAAGAAGACG
Fragment 2	CAAGGAACACCAGGAAGGAGATGAT	ACACACACGCTTTTGATGCTTCT
Fragment 3	ATCCCTTCCAACCTTCCTGAC	GATGACTTCCTCTGCTCTGT
Fragment 4	TGTGCAATCAAGTGACTAAGCTCAAATCG	GTGAAGGAAGATGATTGGATGTTGTTTG
Fragment 5	GGTCTTTTGAAGTGAGATTCTCTGCT	CAGAGTAGTGGAGAGAGGGACAGAT
Fragment 6	CTTATTACACCAACCAGCAAACCTACG	TCAACCAAGTTCAATTCTCGATCCT

**Fig. 1.** Sliding window of *xa13* variants. A sliding window analysis was performed using Dna SP 5.1 software to reveal nucleotide variation among *xa13* variants. Window length: 100 steps; size: 25. Analyzed bp positions were as follows: 1-1,645 (5' end), 1,646-1816 (5' untranslated region), 1,817-4,089 (open reading frame), 4,090-4,523 (3' untranslated region), and 4,524-4,724 (3' end).**Fig. 2.** Sequence variations of the *xa13* gene from different rice varieties. Translation start codon (ATG) is indicated. Arrows represent single nucleotide polymorphisms.

The sequences were aligned using the CLUSTAL W computer program (Thompson *et al.*, 1994) with additional minor modifications based on visual inspection. Alignment of *xa13* gene sequences of the 217 *indica/japonica* rice varieties against the IRBB13 *xa13* sequence (using the ATG translation start codon as a reference) uncovered 19 mutation sites: 2348 A/C, 2212 C/G, 2057 G/A, 2014 C/T, 1865 G/T, 1828 C/T, 1413 C/G, 1211 A/G, 975 C/G, 959 T/C, 809 G/A, 781 T/A, 445 C/T, 380 G/A, 246 T/C, 191 C/A, 116 C/T, -8 C/T, and -119 G/T (Fig. 2). Five and 11 single nucleotide polymorphisms (SNPs) were found in three exons (numbers 3, 4, and 5) and three introns (numbers 1, 2, and 4) respectively. On the basis of the five SNPs in the *xa13* gene coding region, the 217 rice varieties were distributed into six distinct haplotype groups. In addition, a G/A polymorphism in exon 5 resulted in a threonine to alanine substitution at amino acid position 238

Table 2. Protein variation among five *xa13* coding sequence variants^a.

Protein ^b	Variant ^c	87	194	223	238	288
<i>xa13</i>	N/A	A	Y	A	T	A
I	1-5	A	Y	A	A	A

^a Different amino acids and their positions, the letter code used for each amino acid. Number indicates a position of amino acid in the *xa13* protein (ABD78942.1) and bold letters indicate differences in amino acid in comparison with the *xa13* protein.

^b Groups of *xa13* variants based on amino acid sequences.

^c Groups of *xa13* variants based on nucleotide (DQ421394); N/A = not applied.

Variety	S1413	S1828	S2014	S2057	S2212	Protein haplotype	Frequency
Long Grain (35023)	G	C	C	A	C	PH1	11
Nannong 2159	C	C	C	A	C	PH1	1
NGA YA PAUK	C	C	T	G	C	PH2	1
Basmati	C	T	C	G	C	PH2	121
Basmati 370	C	C	C	G	C	PH2	80
Naun Sakony	C	T	C	G	G	PH2	3

Fig. 3. Protein haplotypes based on five exonic single nucleotide polymorphisms in the *xa13* gene of 217 diverse rice varieties.

(Table 2). Alignment of the deduced amino acid sequences supported the existence of two XA13 protein haplotypes (Fig. 3). Analysis of *xa13* gene sequences of the 217 *japonica* and *indica* varieties did not support the existence of two distinct *xa13* haplotypes. This result suggests that natural occurring alleles of *xa13* in the two subspecies do not have independent origins.

In conclusion, this study confirmed sequence variation and haplotypes of the bacterial blight resistance gene *xa13* in rice. Six haplotype groups were developed from the five SNPs in the *xa13* gene coding region. The haplotypes of *xa13* did not segregate in *japonica* and *indica*. The nucleotide diversity of the promoter region was higher than that of either the *xa13* ORF or the 3' region. Moreover, some sites such as a 252 bp insertion in the -69 bp to -86 bp region of the promoter was only detected in three varieties with high BB resistance. Further studies regarding the association of the *xa13* haplotypes with BB resistance/susceptibility are currently under way.

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