Targeting the Recessive Rice Gene, *xa13*, for Bacterial Blight Resistance to a 14.8-kb DNA Fragment

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Abstract

Bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most serious diseases of rice. About 30 bacterial blight resistance (*R*) genes (21 dominant *R* genes and 9 recessive ones) in rice have been identified. These *R* genes are known to act in a gene-for-gene manner and are the main sources for genetic improvement of rice for resistance to *Xoo*. Three cloned dominant *R* genes for resistance to *Xoo* belong to either LRR receptor kinase-like proteins or the NBS-LRR type, but little is known about the recessive *R* genes. To clone and characterize the recessive *R* genes at the molecular level, we fine-mapped a recessive gene, *xa13*, for bacterial blight resistance to a DNA fragment of 14.8 kb using map-based cloning strategy. Sequence analysis of this fragment indicated that this region contained only one complete open reading frame. These results will greatly facilitate the isolation and characterization of *xa13*.

Media summary

Fine mapping of *xa13* gene for bacterial blight resistance will facilitate the isolation and characterization of this recessive gene.

Keywords

Bacterial blight, recessive resistance

Introduction

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most serious diseases of rice. More than 20 BB resistance (*R*) genes, included 9 recessive *R* genes, have been identified from cultivated rice and its wild relatives, or induced by mutagenesis (Lin et al. 1996; Nagato and Yoshimura 1998; Zhang et al. 1998; Khush and Angeles 1999; Chen et al. 2002; Lee et al. 2003; Yang et al. 2003). The dominant *R* genes included *Xa1* to *Xa28*. Six of the recessive *R* genes, *xa5*, *xa8*, *xa13*, *xa24*, *xa26* and *xa28*, occur naturally and confer race-specific resistance; the other 3, *xa15*, *xa19* and *xa20*, are created by mutagenesis and each confers a wide spectrum of resistance to *Xoo* (Ogawa 1996; Lee et al. 2003). To date, only three dominant *R* genes for BB resistance, *Xa1*, *Xa21* and *Xa26*, have been cloned. *Xa21* and *Xa26* encode leucine-rich repeat (LRR) receptor kinase-like proteins, while *Xa1* encodes a nucleotide-binding site (NBS)-LRR protein (Song et al. 1995; Yoshimura et al. 1998; Sun et al. 2004).

The products of dominant *R* genes are presumed to function as receptors, which interact directly or indirectly with pathogen elicitors to initiate hypersensitive responses (Dangl and Jones. 2001). However, recessive *R* genes appear to function differently from most known dominant *R* genes that encode conserved domains. The products of 3 characterized recessive *R* genes, *mlo*, *edr1* and *RRS1-R*, are all different. The dominant *Mlo* encode a transmembrane protein and *EDR1* encodes a MAPKK kinase; both of the dominant proteins act as negative regulators of the defense response (Frye et al. 2001; Kim et al. 2002). Thus *mlo* and *edr1* are the loss-function mutants. Another recessive *R* gene *RRS1-R* encodes a novel NBS-LRR-WRKY protein, which is presumed to function in the nucleus (Lahaye. 2002).

The recessive *R* gene *xa13* specifically confers resistance to the Philippine *Xoo* race 6 that is compatible with most of the BB resistance genes. The *xa13* was first characterized in the rice variety BJ1 and mapped on the long arm of rice chromosome 8 (Ogawa et al.1987; Zhang et al. 1996; Sanchez et al.1999). Unlike other recessive gene for BB resistance, such as *xa5* showing partial dominance to the avirulent *Xoo* races, *xa13* is a completely recessive gene and can strongly interact with *xa5*, *Xa4* and *Xa21* (Li et al. 2001). Small-scale gene expression studies and SSH cDNA library analysis revealed that some defense-responsive genes activated in *xa13*-mediated resistance is not involved in dominant *R* genes (*Xa4*, *Xa10* and *Xa26*) mediated resistance (Zhou et al. 2002; Wen et al. 2003; Chu et al. 2004). These results indicate that *xa13* may function differently from other *R* genes in initiating resistance against *Xoo*. Thus, molecular cloning of *xa13* gene would greatly enhance our understanding of the defensive system in rice. We report here the fine mapping of *xa13* to a 14.8-kb DNA fragment through map-based cloning strategy.

Material and methods

Mapping population and disease evaluation

Two F_2 populations, consisting of 6000 and 1972 individuals derived from a cross between susceptible rice line IR24 and its near-isogenic line IRBB13 carrying *xa13*, were evaluated for resistance to BB in 2000 and 2002, respectively. At booting stage, five of the uppermost fully expanded leaves of each plant were inoculated with Philippine race 6 (PXO99) of *Xoo* by the leaf-clipping method (Kauffman et al. 1973). Reaction to the pathogen was evaluated 21 days after inoculation.

Construction of the physical map covering xa13 region

A BAC library constructed with genomic DNA from rice cultivar Minghui 63 (*indica*) with an average insert length of 150 kb and a coverage equivalent to nine rice genomes (Peng et al. 1998) was used for the construction of the physical map.

DNA sequencing

A shotgun approach was used to determine the nucleotide sequence of BAC clones. The M13 universal forward and reverse primers and the BigDye Terminator Cycle Sequencing v2.0 kit (Applied Biosystems, Forster City, CA, USA) were used for sequencing. Sequences were assembled using the computer program Sequencher 4.1.2 (Gene Codes Coporation, Ann Arbor, MI, USA).

Sequence analysis

DNA sequence similarity analysis was performed using Blastn and Blastx programs (Altschul et al.1997). The gene was predicted by using Genscan program (Burge and Karlin, 1997) and Blast analysis against rice EST database (http://bioinformatics.hzau.edu.cn).

Results and discussion

Physical mapping of xa13 with BACs

According to the genetic mapping results (Zhang et al. 1996; Sanchez et al. 1999), the *xa13* gene was located on the long arm of rice chromosome 8 flanked by two RFLP markers, RG136 and R2027. Our results indicated that a PCR marker, E6a, located between RG136 and *xa13*, and a RFLP marker, S14003, flanked R2027. Thus, *xa13* gene located between E6a and S14003. A physical map covering the region flanked by markers E6a and S14003 was constructed by chromosome walking using Minghui 63 BAC clones (Fig. 1). This map composed of 5 (22E06, 23P23, 30A21, 39N05 and 44E01) overlapping BAC clones. One clone, 44E01, showed sequence homology with two BAC clones from rice variety IR64, IR64a and IR64b locating in the *xa13* region as reported by Sanchez et al. (1999).

DNA sequencing

Both Minghui 63 and IR64 are susceptible to *Xoo* strain PXO99. Thus they carry the allele of *xa13* or the dominant gene *Xa13*. The two BAC clones, IR64a and IR64b from IR64, were sequenced. IR64a was about 90 kb in size and IR64b was 66.6 kb.

Fine mapping of xa13

The F_2 plants were examined using markers flanking *xa13* to detect recombination events occurred between *xa13* locus and markers. Twenty-six individuals from the 6000 F_2 population grown in 2000 showed recombination between *xa13* and marker E6a (Fig. 1). Six shotgun clones (RP3, RP4, RP5, RP7, RP8 and RP10) from IR64a and IR64b, two SSR markers (SR6 and SR11) and a PCR marker (ST9) were further used to screen the 26 recombinant individuals. Markers RP3, RP4, RP5, SR6 and RP7 detected 15 to 1 recombination events and the rest 4 markers co-segregated with *xa13* (Fig. 1).

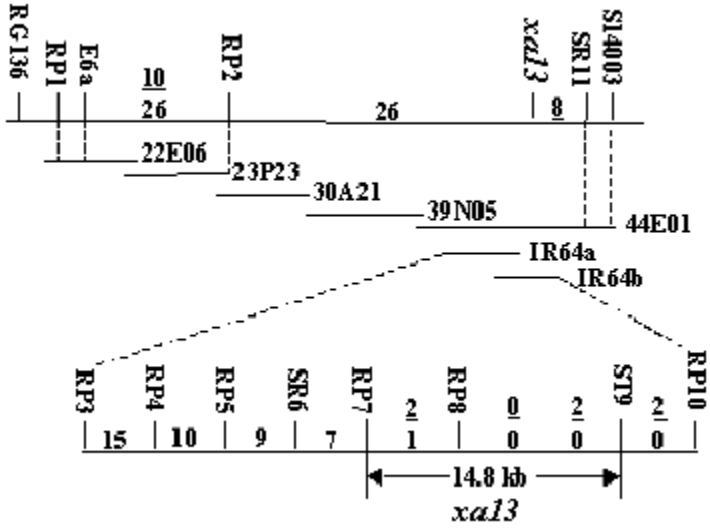

Figure 1. A contig map covering the *xa13* region. The long horizontal line indicates the region containing *xa13* locus. The short horizontal lines represent BAC clones. The numbers between molecular markers indicate the number of recombination events detected between *xa13* locus and corresponding marker. The underlined numbers represent recombination events detected from the F_2 population grown in 2002. Other numbers represent recombination events detected from he F_2 population grown in 2000. The dashed vertical lines between markers and BAC clones indicate that hybridization between markers and BAC clones was verified.

In 2002, 1972 F_2 plants were also examined with markers flanking *xa13*. Ten recombinant individuals were identified using marker E6a flanking one side of *xa13* locus (Fig. 1). Recombination was further detected occurring between RP7 and *xa13* in 2 of the 10 plants. But marker RP8 co-segregated with *xa13* in the 10 plants detected by E6a. The marker SR11 on the other side of *xa13* locus detected 8 recombinant individuals in the 1972 F_2 plants. Two recombination events occurring between ST9 and *xa13* loci were further identified in 2 of the 8 plants detected by SR11. Combining the results obtained from analysis of 36 and 8 F_2 recombinants detected by E6a and SR11, respectively, the region containing the *xa13* locus was narrowed down to a fragment flanked by RP7 and ST9. The sequences of BAC clones IR64a and IR64b revealed that this fragment was only 14.8 kb in length.

Conclusions

We have targeted a recessive gene, *xa13*, for bacterial blight resistance to a 14.8 kb DNA fragment. Sequence analysis of this fragment indicated that this region contained only one complete open reading frame. These results will greatly facilitate the isolation and characterization of this gene.

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