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# Fine mapping of *Xa2*, a bacterial blight resistance gene in rice

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**Abstract**

The rice bacterial blight resistance gene, *Xa2*, confers resistance to T7147 of the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae*. It is located on the long arm of chromosome 4. Here, we report the fine mapping of *Xa2* by genetic recombination analysis with simple sequence repeat (SSR) markers according to the genome sequence. Two $F_2$ populations are constructed to localize *Xa2*. In a primary analysis with 136 random $F_2$ plants of Zhenzhuai/IRBB2, it was found that *Xa2* was located in approximately 20 cM region. To accurately determine the locus of *Xa2*, 120 new SSR markers were developed in this region by screening the sequence. Twelve new SSR markers were successfully used in genetic recombination analysis in IR24/IRBB2 population, while 20 in ZZA/IRBB2 population. We found that the nearest SSR markers to *Xa2* are HZR950-5 and HZR970-4, which cover approximately 190-kb region. The sequence analysis of this 190-kb region revealed the presence of a homologous sequence of leucine rich repeat (LRR)-kinase. These results are very useful for transferring or pyramiding *Xa2* by molecular marker-assistant selection in rice breeding programs and for cloning *Xa2* by map-based cloning in combination with a long-range PCR strategy.

## Introduction

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most serious diseases in rice worldwide. Developing resistant cultivars is generally considered to be the most effective and economical means of controlling this disease (Gnanamanickam et al. 1999). Numerous major genes which confer resistance to various *Xoo* strains have been identified and named in a series from *Xa1* to *Xa28*. More than 10 BB resistance genes have been mapped on various chromosomes, especially on chromosomes 4 and 11 (Kinoshita 1995; Lin et al. 1996; Zhang et al. 1998; Chen et al. 2002; Lee et al. 2003; Yang et al. 2003; Gu et al. 2004). Some of the mapped resistance genes were utilized in transferring or pyramiding with molecular marker-assisted selection (MAS) rice breeding programs (Yoshimura et al. 1995; Huang et al. 1997; Chen et al. 2000).

For rice, a cereal model plant, high-density genetic linkage maps are available including Simple Sequence Repeat (SSR) markers (Causse et al. 1994; Harushima et al. 1998; Temnykh et al. 2000). The emergence of complete genome sequences in rice offers new opportunities to increase the density of locus-specific, polymorphic markers for high-resolution genetic analysis (McCouch et al. 2002).

Flanking molecular markers, which are physically very close to the target gene, are then used to obtain the candidate clone by a long-range polymerase chain reaction (LR-PCR) strategy (Song et al. 2003). Map-based cloning is now an efficient approach to isolate functional genes if they can be precisely mapped. Three rice bacterial blight resistant genes (*Xa1*, *Xa21*, and *Xa26*) were cloned by map-based cloning strategy (Song et al. 1995; Yoshimura et al. 1998; Sun et al. 2004).

The BB resistance gene, *Xa2*, confers specific resistance to T7147 (Japan *Xoo* race 2). It was identified and named by Sakaguchi (1967). Furthermore, *Xa2* was mapped on chromosome 4, linked to *Xa1* with a recombination frequency of 2∼16% (Yoshimura et al. 1994). IRBB2, IR24s near isogenic line (NIL) carrying *Xa2*, was developed at the International Rice Research Institute (IRRI) and the Tropical Agriculture Research Center (TARC) (Ogawa et al. 1988, 1991). In this paper, we report the fine mapping of *Xa2* in an effort to set up a MAS system of *Xa2* and clone *Xa2* by map-based cloning in combination with a LR-PCR strategy. We have identified a number of SSR markers tightly linked to *Xa2* and the nearest two of these cover approximately 190 kb in length.

## Materials and methods

### *Pathogen strains and disease evaluation*

*Xoo* strain T7147 (provided by T. Ogawa) was used to assay resistance in two mapping populations. The bacterial strain was seeded on a potato semi-synthetic agar medium (Wakimoto 1954) and incubated at 28 °C for 3 days. Inoculum was prepared by suspending the bacterial mass in sterilized water at a concentration of approximately $6\times10^8$ cells per ml (measured by the barium sulfate turbidimetry method).

Rice seedlings were transplanted to the disease nursery 30 days after sowing. The space between plants in a row was 12 cm, and the rows were 24 cm apart. In all the experiments, the parents and $F_1$ populations were replicated twice, with 10 plants per replication. The $F_2$ populations were tested on the basis of individual plants. At the booting stage (approximately 40 days after transplanting), 5–7 of the uppermost fully expanded leaves of each plant were inoculated by the leaf clipping method, in which the leaf was cut with a pair of scissors dipped in the bacterial suspension (Kauffman et al. 1973). For disease scoring, the longest lesion of two or three undamaged leaves per plant was measured 20 days after inoculation. A plant was classified as resistant (average lesion length <6.0 cm) and susceptible (average lesion length >6.0 cm).

### *Plant material and mapping populations*

Zhenzhuai (ZZA, susceptible to all *Xoo* strains, *Oryza sativa* ssp. *Indica*), IR24 (susceptible to all *Xoo* strains, *Oryza sativa* ssp. *Indica*), and IRBB2 (IR24s NIL, carrying *Xa2*) were used as parents of mapping populations. Two $F_2$ populations were constructed for the fine mapping of *Xa2*. The first population consisted of 241 $F_2$ plants derived from a cross between IR24 and IRBB2. The second population consisted of 1,579 $F_2$ plants derived from a cross between ZZA and IRBB2, in which 136 random plants were used to construct a linkage map flanking the *Xa2* locus and to confirm inheritance of BB resistance in IRBB2.

### *Development of SSR*

The sequence of rice chromosome 4, released by the National Center for Gene Research, Chinese Academy of Science (http://www.ncgr.ac.cn/chinese/p2-j.htm), was used for developing SSR markers. SSRSCAN, a computer program designed by Molecular Biology Division, National Key Laboratory of Crop Genetic Improvement (http://redb.croplab.org/modules/redbtools/ssr-scan.php), was used to identify SSR markers in the target sequence. PCR primer pairs flanking SSR markers were automatically selected using the primer3 programmer (Rozen and Skaletsky 2000). All SSR markers were designated 'HZR', and were followed by the last 3 codes of BAC/PAC accession number and a natural number separated by a hyphen ('-') in the same clone.

### *PCR amplification and estimates of polymorphism*

Total DNA was extracted from fresh leaf tissues by CTAB method. The polymerase chain reaction

(PCR) was carried out in a reaction volume of 20 $\mu$l containing 0.2 $\mu$M of each primer, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 1× PCR buffer (50 mM KCl, 10 mM Tris, pH 8.3), 50–100 ng template DNA and 1 unit of *Taq* polymerase. The PCR profile was: 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and finally 5 min at 72°C for the final extension. PCR products were run on 4% polyacrylamide denaturing gels, and marker bands were revealed using silver staining as described by Panaud et al. (1996). The SSR markers showing polymorphism between the resistant and susceptible parents were used to analyze the $F_2$ population.

*Data analysis*

The SSR linkage map flanking the *Xa2* locus was determined by using the data of 136 random $F_2$ plants of ZZA/IRBB2. The genetic distance among SSR markers was evaluated by MAPMAKER3.0 at a LOD threshold of 3.0 (Lincoln et al. 1992). We used recessive class of *Xa2* locus (highly susceptible plant, average lesion length >9.0 cm) to identify the recombinant events between the SSR markers and the locus of *Xa2* because the efficiency of mapping with recessive class is two to three times higher than with a random $F_2$ population per assayed plant (Zhang et al. 1994). Also, it is likely that the disease scores of highly susceptible plants better reflect the genotype at the resistance locus than those with intermediate scores. The linkage index was evaluated as number of recombinants between the SSR markers and the *Xa2* locus.

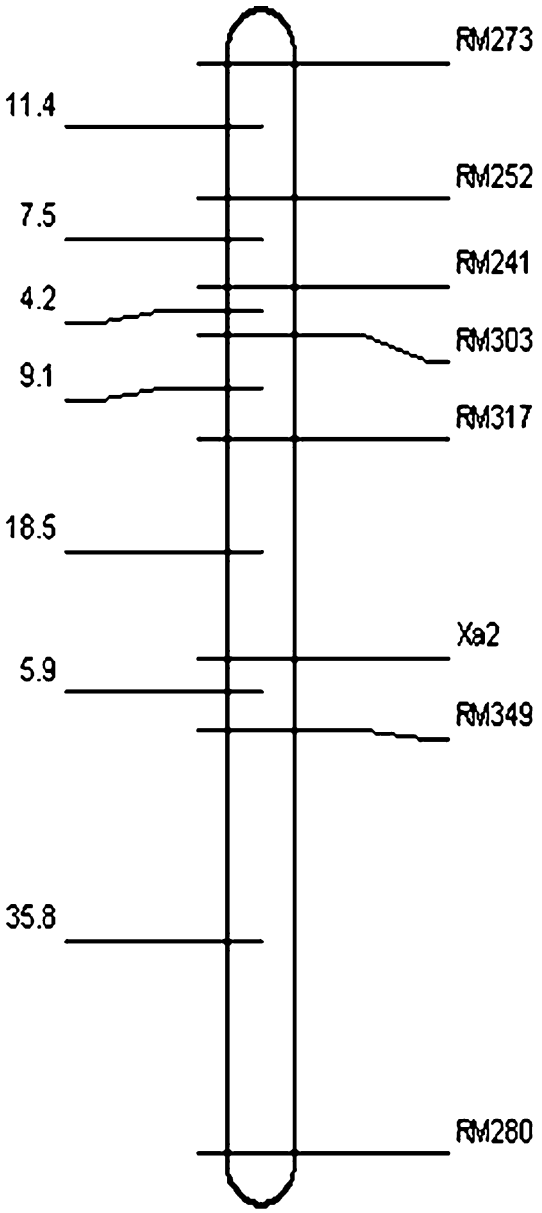


*Figure 1.* The SSR linkage map of the *Xa2* region on rice chromosome 4.

## Results

*Inheritance of bacterial blight resistance in IRBB2*

IRBB2, carrying *Xa2*, is resistant to T7147 with an average lesion length of 0.5 ± 0.1 cm, while IR24 and ZZA are susceptible with an average lesion length of 17.0 ± 3.0 and 18.5 ± 3.5 cm, respectively. All the $F_1$ plants of IR24/IRBB2 and ZZA/IRBB2 are resistant to T7147 (data not shown). Segregation of resistant and susceptible plants (104 resistant plants, 32 susceptible plants) fit a 3:1 ratio in the 136 random $F_2$ plants ($\chi^2 = 0.15686$, $p > 0.5$), which indicates that the resistance of IRBB2 to T7147 is only controlled by one dominant gene, *Xa2*. Twenty-five highly susceptible plants were obtained from 241 $F_2$ plants of IR24/IRBB2, while 262 from 1579 $F_2$ plants of ZZA/IRBB2.

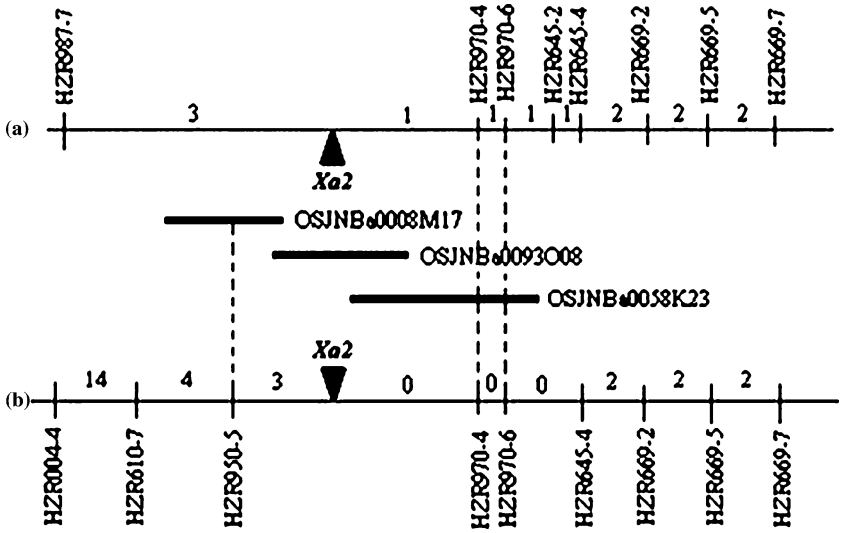


*Figure 2*. The genetic and physical map of resistance gene *Xa2*. HZR987-7, etc. are SSR markers. OSJNBa0008M17, OSJNBa0093O08 and OSJNBa0058K23 are BAC clones released by National Center for Gene Research, CAS. The numbers in the map mean the amount of recombinant between SSR marker and *Xa2* locus. (a) The map is constructed with five recombinant events from 241 $F_2$ plants of IR24/IRBB2. (b) The map is constructed with 16 recombinant events from 1579 $F_2$plants of ZZA/IRBB2.

*Linkage analysis of* Xa2

In the region of *Xa1* locus covering approximately 50 cM, a total of 47 SSR markers were selected from the previously reported assays to identify polymorphism between ZZA and IRBB2. Eleven SSR markers showing polymorphism were used to screen the 136 random $F_2$ plants of ZZA/IRBB2. The SSR markers linkage map was determined by analyzing with the Mapmaker program. Two of the closest flanking SSR markers (RM317 and RM349) were linked to *Xa2* with 18.5 and 5.9 cM, respectively (Figure 1).

*Analysis of recombinant events*

We identified the recombinant events between SSR markers and *Xa2* locus according to the genotype of SSR marker from highly susceptible $F_2$ plants. Nineteen recombinant plants were identified from 262 highly susceptible $F_2$ plants of ZZA/IRBB2 according to RM317 and RM349 analysis. Six recombinant plants were identified from 25 highly susceptible $F_2$ plants of IR24/IRBB2 by RM317 and HZR455-7 (Supplementary table 2). Above 19 and 6 recombinant plants were confirmed by identifying the lesion length and SSR marker's genotype of the respective $F_3$ progenies. These recombinant plants were used to fine map *Xa2*.

*Fine mapping of* Xa2

We developed new SSR markers to saturate the genetic map and to further accurately localize *Xa2*. The previous results indicated that RM317 was located on BAC OSJNBa0011J08 (GenBank accession No. AL606624), while RM349 on OSJNBb0079B02 (GenBank accession No. AL606455). New SSR markers were developed based on the available sequence of clones from OSJNBa0011J08 to OSJNBb0079B02. A total of 120 new SSR markers were designed. Among these, 12 new SSR markers show polymorphism between IR24 and IRBB2, while 20 between ZZA and IRBB2 (Supplementary table 1). These SSR markers were used for narrowing down the region containing the *Xa2* locus step by step. The results show that HZR987-7 and HZR970-4 were the closest SSR markers to the *Xa2* locus in the population of IR24/IRBB2, while HZR950-5 and HZR970-4 in the population of ZZA/IRBB2. We found that *Xa2* was localized between HZR950-5 and HZR970-4 (Figure 2), which covers approximately 190-kb according to the sequence released.

## Discussion

In this study, we developed 120 new SSR markers within approximately 20-cM region to map BB resistance gene *Xa2*. The results indicate that *Xa2* is localized in approximately 190-kb long region. It will be useful for establishing the MAS system to transfer *Xa2* or pyramid *Xa2* with other rice resistance genes in breeding program. Sequence analysis of the 190-kb region showed that the sequence is homologous to leucine rich repeat (LRR)-kinase, which is the product of a cloned rice BB resistance gene *Xa21* (Song et al. 1995). To examine this candidate gene (*Xa2*), we obtained the fragment from IRBB2 by LR-PCR strategy and transferred this to IR24 and Taipei 309 (susceptible to all *Xoo* strains, a model cultivar for transformation) by *Agrobacterium* to identify its function.

Many rice BB resistance genes (*Xa1*, *Xa2*, *Xa12*, and *Xa14*) are mapped to long arm of chromosome 4 (Ogawa et al. 1978; Taura et al. 1987; Yoshimura et al. 1994). It has been proposed that a disease resistance locus is made up of many alleles that can recombine to produce a locus with a novel specificity (Pryor 1987; Ronald et al. 1992). It is speculated that duplications and rearrangements in the plant genome may have given rise to the complexity and race specificity observed in many disease resistance loci. It would be very interesting to sequence and compare these regions from different cultivars carrying different resistance genes.

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## References

Causse M.A., Fulton T.M., Cho Y.G., Ahn S.N., Chunwongse J., Wu K.S., Xiao J.H., Yu Z.H., Ronald P.C., Harrington S.E., Second G., McCouch S.R. and Tanksley S.D. 1994. Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138: 1251–1274.

Chen S., Lin X.H., Xu C.G. and Zhang Q.F. 2000. Improvement of bacterial blight resistance of 'Minghui 63', an elite restorer line of hybrid rice, by molecular marker-assisted selection. Crop Sci. 40(1): 239–244.

Chen H., Wang S. and Zhang Q. 2002. New gene for bacterial blight resistance in rice located on chromosome 12 identified from Minghui 63, an elite restore line. Phytopathology 92: 750–754.

Gnanamanickam S.S., Priyadarisini V.B., Narayanan N.N., Vasudevan P. and Kavitha S. 1999. An overview of bacterial blight disease of rice and strategies for its management. Current Sci. 77: 1435–1443.

Gu K., Tian D., Yang F., Wu L., Sreekala C., Wang D., Wang G.L. and Yin Z. 2004. High-resolution genetic mapping of *Xa27*(t), a new bacterial blight resistance gene in rice, *Oryza sativa* L. Theor. Appl. Genet. 108: 800–807.

Harushima Y., Yano M., Shomura A., Sato M., Shimano T., Kuboki Y., Yamamoto T., Lin S.Y., Antonio B.A., Parco A., Kajiya H., Huang N., Yamamoto K., Nagamura Y., Kurata N., Khush G.S. and Sasaki T. 1998. A high-density rice genetic linkage map with 2275 markers using a single $F_2$ population. Genetics 148: 479–494.

Huang N., Angeles E.R., Domingo J., Magpantay G., Singh S., Zhang G., Kumaravadivel N., Bennett J. and Khush G.S. 1997. Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theor. Appl. Genet. 95: 313–320.

Kinoshita T. 1995. Report of committee on gene symbolization, nomenclature and linkage groups. Rice Genet. Newslett. 12: 9–153.

Kauffman H.E., Reddy A.P.K., Hsieh S.P.Y. and Merca S.D. 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant Disease Rep. 57: 537–541.

Lee K.S., Rasabandith S., Angeles E.R. and Khush G.S. 2003. Inheritance of resistance to bacterial blight in 21 cultivars of rice. Phytopathology 93: 147–152.

Lin X.H., Zhang D.P., Xie Y.F., Gao H.P. and Zhang Q. 1996. Identifying and mapping a new gene for bacterial blight resistance in rice based on RFLP markers. Phytopathology 86: 1156–1159.

Lincoln S., Daly M. and Lander E. 1992. Constructing genetic maps with Mapmaker/Exp 3.0. 3rd ed. Whitehead Institute Technical Report, Cambridge, Massachusetts.

McCouch S.R., Teytelman L., Xu Y., Lobos K.B., Clare K., Walton M., Fu B., Maghirang R., Li Z., Xing Y., Zhang Q., Kono I., Yano M., Fjellstrom R., Declerck G., Schneider D., Cartinhour S., Ware D. and Stein L. 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res. 9: 199–207.

Ogawa T., Morinaka T., Fujii K. and Kimura T. 1978. Inheritance of resistance of rice varieties Kogyoku and Java 14 to bacterial group V of *Xanthomonas oryzae*. Ann. Phytopathol. Soc. Japan 44(2): 137–141.

Ogawa T., Yamamoto T., Khush G.S., Mew T.W. and Kaku H. 1988. Near-isogenic lines as international differentials for resistance to bacterial blight of rice. Rice Genet. Newslett. 5: 106–107.

Ogawa T., Yamamoto T., Khush G.S. and Mew T.W. 1991. Breeding of near-isogenic lines of rice with single genes for resistance to bacterial blight pathogen (*Xanthomonas campestris* pv. *oryzae*). Japan J. Breed. 41: 523–529.

Panaud O., Chen X. and McCouch S.R. 1996. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). Mol. Gen. Genet. 252: 597–607.

Pryor T. 1987. The origin and structure of fungal disease resistance genes in plants. Trends Genet. 3: 157–161.

Ronald P.C., Albano B., Tabien R., Abenes L., Wu K., McCouch S. and Tanksley S.D. 1992. Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. Mol. Gen. Genet. 236: 113–120.

Rozen S. and Skaletsky H. 2000. Primer3 on the WWW for general users and for biologist programmers. Methods Mol. Biol. 132: 365–386.

Sakaguchi S. 1967. Linkage studies on the resistance to bacterial leaf blight, *Xanthomonas oryzae* (Uyeda et Ishiyama) DOWSON, in rice. Bull. Natl. Inst. Agr. Sci. Ser. D 16: 1–18.

Song J., Bradeen J.M., Naess S.K., Raasch J.A., Weilgus S.M., Haberlach G.T., Liu J., Kuang H., Austin-Phillips S., Buell C.R., Helgeson J.P. and Jiang J. 2003. Gene RB cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. Proc. Natl. Acad. Sci. USA 100: 9128–9133.

Song W.Y., Wang G.L., Chen L.L., Kim H.S., Pi L.Y., Holsten T., Gardner J., Wang B., Zhai W.X., Zhu L.H., Fauquet C. and Ronald P. 1995. A receptor kinase-like protein encoded by the rice disease resistance gene *Xa21*. Science 270: 1804–1806.

Sun X.L., Cao Y.L., Yang Z.F., Xu C.G., Li X.H., Wang S.P. and Zhang Q.F. 2004. *Xa26*, a gene conferring resistance to *Xanthomonas oryzae* pv. *oryzae* in rice, encodes an LRR receptor kinase-like protein. Plant J. 37(4): 517–527.

Taura S., Ogawa T., Tabien R.E., Khush G.S., Yoshimura A. and Omura T. 1987. The specific reaction of Taichung Native 1 to Philippine race of bacterial blight and inheritance of resistance to race 5 (PX0112). Rice Genet. Newslett. 4: 101–102.

Temnykh S., Park W.D., Ayres N., Cartinhour S., Hauck N., Lipovich L., Cho Y.G., Ishii T. and McCouch S.R. 2000. Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor. Appl. Genet. 100: 697–712.

Wakimoto S. 1954. The determination of the presence of *Xanthomonas oryzae* by the phage technique. Sci. Bull. Fac. Agric. Kyushu Univ. 14: 485–493.

Yang Z., Sun X., Wang S. and Zhang Q. 2003. Genetic and physical mapping of a new gene for bacterial blight resistance in rice. Theor. Appl. Genet. 106: 1467–1472.

Yoshimura S., Kurata N., Sasaki T. and Yoshimura A. 1994. Genetic analysis of bacterial blight resistance genes in rice by using molecular markers. Recent Adv. Breed. 36: 33–36.

Yoshimura S., Yoshimura A., Iwata N., McCouch S.R., Abenes M.L., Baraoidan M.R., Mew T.W. and Nelson R. 1995. Tagging and combing bacterial blight resistance genes in rice using RAPD and RFLP markers. Mol. Breed. 1: 375–387.

Yoshimura S., Yamanouchi U., Katayose Y., Toki S., Wang Z.X., Kono I., Kurata N., Yano M., Iwata N. and Sakaki T. 1998. Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. Proc. Natl. Acad. Sci. USA 95: 1663–1668.

Zhang Q., Lin S.C., Zhao B.Y., Wang C.L., Yang W.C., Zhou Y.L., Li D.Y., Chen C.B. and Zhu L.H. 1998. Identification and tagging a new gene for resistance to bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) from *O. rufipogon*. Rice Genet. Newslett. 15: 138–142.

Zhang Q., Shen B.Z., Dai X.K., Mei M.H., Saghai Maroof M.A. and Li Z.B. 1994. Using bulked extremes and recessive classes to map genes for photoperiod-sensitive genic male sterility in rice. Proc. Natl. Acad. Sci. USA 91: 8675–8679.