Introduction

Rice (Oryza sativa L.) is a cereal crop of the family Poaceae. It has been gathered, cultivated, and consumed by many people worldwide. In fact, it is the world’s most important food crop and a primary source of food for more than half the world’s population (Khush 2005). Unfortunately, its production potential is being reduced severely by several rice diseases. Bacterial blight (BB) caused by the Xanthomonas oryzae pv. oryzae (Xoo) pathogen is a chief factor limiting rice productivity worldwide because of its high epidemic potential (Khan et al. 2014, Verdier et al. 2012, Xia et al. 2012).

As a vascular disease that results in systemic infection, BB produces tannish-grey to white lesions along leaf veins (Mew 1987, Mew et al. 1993). Most commonly, plants are affected at the maximum tillering stage. Yields are reduced by 10–20%. Infection at the tillering stage can engender total crop losses (Mew et al. 1993). Actually, BB is prevalent in both tropical and temperate climates. It has been reported in all rice growing regions worldwide except North America (Niño-Liu et al. 2006, Ou 1985).

Developing resistant cultivars is generally regarded as the most effective and economical means of controlling this disease (Khan et al. 2014, Mew et al. 1993, Ogawa and Khush 1989). About 40 genes conferring resistance against various races of Xoo have been identified in both cultivated rice and wild relatives of rice, and have been derived from artificial mutation induction (http://www.shigen.nig.ac.jp/rice/oryza/loci/loci?lang=en). However, because of the rapid changes in the pathogenicity of Xoo and the emergence of new races, resistance genes break down (Khan et al. 2014, Mew 1987, Xia et al. 2012). To solve this problem, a search for new Xoo-resistance genes has been conducted. Taura et al. (1991b, 1992) identified Xoo-resistant mutant genes xa19 and xa20 using N-methyl-N-Nitrosourea (MNU) mutagen. These genes are resistant to all Japanese and Philippines races. We have identified new mutant named ‘XM14’, which is resistant to all Japanese Xoo races. This study was conducted for identification, and for genetic and linkage analysis of the Xoo resistance gene in XM14.

Identification and linkage analysis of a new rice bacterial blight resistance gene from XM14, a mutant line from IR24

Constantine Busungu1), Satoru Taura2), Jun-Ichi Sakagami3) and Katsuyuki Ichitani*3)

1) United Graduate School of Agricultural Sciences, Kagoshima University, 1-21-24 Korimoto, Kagoshima, Kagoshima 890-0065, Japan
2) Institute of Gene Research, Kagoshima University, 1-21-24 Korimoto, Kagoshima, Kagoshima 890-0065, Japan
3) Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima, Kagoshima 890-0065, Japan

Bacterial blight caused by Xanthomonas oryzae pv. oryzae (Xoo) is a chief factor limiting rice productivity worldwide. XM14, a rice mutant line resistant to Xoo, has been obtained by treating IR24, which is susceptible to six Philippine Xoo races and six Japanese Xoo races, with N-methyl-N-nitrosourea. XM14 showed resistance to six Japanese Xoo races. The F2 population from XM14 × IR24 clearly showed 1 resistant : 3 susceptible segregation, suggesting control of resistance by a recessive gene. The approximate chromosomal location of the resistance gene was determined using 10 plants with shortest lesion length in the F2 population from XM14 × Koshihikari, which is susceptible to Japanese Xoo races. DNA marker-assisted analysis revealed that the gene was located on chromosome 3. IAS16 line carries IR24 genetic background with a Japonica cultivar Asominori segment of chromosome 3, on which the resistance gene locus was thought to be located. The F2 population from IAS16 × XM14 showed a discrete distribution. Linkage analysis indicated that the gene is located around the centromeric region. The resistance gene in XM14 was a new gene, named XA42. This gene is expected to be useful for resistance breeding programs and for genetic analysis of Xoo resistance.

Key Words: Oryza sativa, Xanthomonas oryzae pv. oryzae, mutation, DNA marker, resistance by a recessive gene.
Materials and Methods

Bacterial races and plant materials for genetic analysis

*Xoo* are differentiated into many races according to virulence and origin. Races used for this study were six Japanese races: race I (strain T7174), race IIA (strain T7147), race IIB (strain H9387), race III (strain T7133), race IV (strain H75373), and race V (strain H75304). In addition, a race from the Philippines was used: race 5 (strain PXO 112).

The Indica rice cultivar 'IR24' was released in 1972 by the International Rice Research Institute (IRRI). IR24 is susceptible to six Philippines *Xoo* races (race 1 (strain PXO 61), race 2 (strain PXO 86), race 3 (strain PXO 79), race 4 (strain PXO 71), race 5 (strain PXO 112), and race 6 (strain PXO 99) (Taura et al. 1991b, 1992). Moreover, it is susceptible to the six Japanese *Xoo* races above (Ogawa and Yamamoto 1987). We induced mutation to IR24, as described by Taura et al. (1991a): We soaked rice spikelets of IR24 in 1 mM of MNU solutions for 45 min at 8, 10, and 12, 14, 16, 18 and 20 hr after flowering. M1 plants were selfed to produce M2 generation. We selected a M2 plant resistant to Philippine *Xoo* race 5 at IRRI, Los Baños, Philippines in 1988. The M3 line derived from the resistant M2 plant was fixed for *Xoo* resistance. The progeny of the M3 line was named XM14. Thereafter, XM14 was brought to Japan. Then studies of XM14 resistance to Japanese *Xoo* races were conducted. Preliminary results showed that XM14 is resistant to the six Japanese *Xoo* races described above. ‘Koshihikari’, a popular Japonica rice cultivar, is cultivated in Japan as well as Australia and the United States. This cultivar is known to be susceptible to all Japanese *Xoo* races (Noda and Ohuchi 1989).

IAS lines are one of the sets of reciprocal chromosome segment substitution lines (CSSSLs) between a Japanese Japonica cultivar ‘Asominori’ and IR24 (Kubo et al. 2002). The graphical genotypes of IAS lines are obtainable at http://www.shigen.nig.ac.jp/rice/oryzaabase/strain/recombinant/genotypeIAS. Among them, the IAS16 line carries IR24 genetic background with Asominori chromosomal segment of chromosome 3, on which resistance gene of XM14 was thought to be located from the initial mapping (see Results). Asominori is resistant to Japanese *Xoo* races I and V while susceptible to races II, III, and IV (Kaku and Kimura 1989). Our preliminary analysis showed that IAS16 is susceptible to the six Japanese *Xoo* races above.

XM14, IR24, IAS 16, and Koshihikari were tested for their reactions to the six Japanese *Xoo* races. They had been tested separately before, and were tested under the same condition in 2014. Each *Xoo* race was inoculated to six plants from each line. XM14 was crossed with IR24 to ascertain the number of gene(s) and dominance involved in *Xoo* resistance using 216 F2 plants. XM14 was also crossed with Koshihikari. A total of 237 F2 plants from the cross between XM14 and Koshihikari were subjected to preliminary linkage analysis to ascertain the approximate chromosomal location of the resistance gene of XM14. The Results section of this report presents a description that this population showed continuous distribution of LL, probably because of diverse genetic background attributable to the Indica–Japonica cross. To minimize the genetic ‘noise’, the 194 F2 plants from the cross between XM14 and IAS16 were also subjected to linkage analysis because both lines share the IR24 genetic background. During the following season, F3 lines from selected F2 plants from the same cross were grown to confirm the genotypes of the resistance gene in XM14.

F2 plants from the cross between XM14 and IR24 had been planted before, with the preliminary result that XM14 carries a recessive resistance gene. They were planted again in 2014, increasing the number of plants for confirmation for the previous result. F3 plants from the cross between XM14 and Koshihikari were planted in 2012. F3 plants from the cross between XM14 and IAS16 were planted in 2014. F3 lines from the same cross were planted in 2015. Germinated seeds of segregating populations and parental lines were sown in seedling boxes in a greenhouse in May in respective years. About two weeks after sowing, seedlings were transferred out of the greenhouse. About one month after the sowing date, they were transplanted to a paddy field in the experimental farm of the Faculty of Agriculture, Kagoshima University, Kagoshima, Japan. Along with the respective segregating populations, 5 to 10 plants from each parental line were planted.

Inoculation of *Xoo* and scoring

*Xoo* inoculum was prepared and cultured using potato semi-synthetic agar media (Wakimoto 1954) and was incubated at 28°C for 48 hr. The inoculum was then diluted with distilled water. The absorbance was adjusted to A = 0.05 (620 nm) using a spectrophotometer. This value corresponds to the concentration of about 10⁶ colony forming units per milliliter (cfu/ml), which normally provide optimum *Xoo* infection to the host using the clipping method (Kauffman et al. 1973). *Xoo* was inoculated using the clipping method during booting to the flowering stage. Resistance of plants to *Xoo* was scored by the mean lesion length (LL) of three leaves from each plant using a ruler 18 days after *Xoo* inoculation. Scoring of LL of F2 lines was based on visual observation: LL longer than 3 cm was scored as susceptible, whereas that shorter than 3 cm was scored as resistant.

DNA markers and linkage analysis

PCR-based SSR and Insertion/deletion (Indel) markers were used for linkage analysis. DNA was extracted according to the method described by Dellaporta et al. (1983) with some modifications. Polymerase chain reaction (PCR) mixture (5 μL) consisted of 10 ng genomic DNA, 200 μM dNTPs, 0.2 μM of each primer, 0.25 U of Taq polymerase (Amplitaq Gold; Applied BioSystems, CA, USA), and 1 × buffer containing MgCl₂. PCR conditions were: 95°C for 5 min, 35 cycles 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, 55°C for 30 s and 72°C...
for 30 s with subsequent final extension at 72°C for 7 min. After PCR products were separated in 10% polyacrylamide gels, they were stained with ethidium bromide and visualized with ultraviolet light (GelDoc-It® TS Imaging System; UVP, CA, USA).

For initial linkage analysis using the cross between Koshihikari and XM14, we used 113 published SSR markers and Insertion/deletion (Indel) markers, most with primer information derived from reports by Ichitani et al. (2014), IRGSP (2005), McCouch et al. (2002), Panaud et al. (1996), and Rice Genome Research Program (http://rgp.dna.affrc.go.jp/E/publicdata/caps/index.html).

As the linkage analyses progressed, the target regions of the Xoo resistance gene were narrowed. No published DNA markers were present there. Therefore, we developed new PCR-based DNA markers (Table 1). We used Indel information released by Xu et al. (2012) or searched for Indel polymorphism (5–50 bp difference) between a Japonica cultivar ‘Nipponbare’ (IRGSP 2005, Kawahara et al. 2013) and an Indica cultivar ‘93-11’ (Gao et al. 2013, Yu et al. 2002), and/or an Indica cultivar ‘HR12’. *Oryza sativa* (rice) Nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&PROG_DEF=blastn&BLAST_PROG_DEF=megaBlast&BLAST_SPEC=OGP__4530__9512) was used for Indel information. BLAST searching optimized for highly similar sequences was done using a one thousand to ten thousand base Nipponbare sequence (Os-Nipponbare-Reference-IRGSP-1.0) as the query and 93-11 sequence (GCA_000004655.1) or HR12 sequence (GCA_000725085) as the subject. Uniqueness of the DNA sequences surrounding Indel was confirmed using BLAST with BLASTScope in Oryzabase (Yamazaki et al. 2010, http://www.shigen.nig.ac.jp/rice/oryzabaseV4/blast/search). Primers surrounding Indels were designed using Primer 3 (Untergrasser et al. 2012). Linkage map involving 16 DNA markers on chromosome 3 was constructed using software (AntMap; Iwata and Ninomiya 2006). The Kosambi function was used to estimate the map distances (Kosambi 1944).

### Table 1. Primer sequences of DNA markers designed or redesigned for linkage analysis of XA42 gene

| Marker name  | Kind of DNA marker | Primer sequence | Location on IRGSP 1.0 pseudomolecule chromosome 3 |
|--------------|--------------------|-----------------|-----------------------------------------------|
| KGC3_15.36   | INDEL F            | ATTTCCGATGGATAGATAATTGCTCAA TCTAGTTGGACAGACAGACGTA | 15369490–15369606 This study |
| KGC3_15.39   | INDEL F            | GCCGTGCAAGAATATCTGCAAATATCT | 15392101–15392223 This study |
| KGC3_15.57   | INDEL F            | TCAATTAGCTGCTGAGACCGCATC GACATGTCAGGAAGAATGCTCC | 15571005–15571205 This study |
| KGC3_15.7    | INDEL F            | CACGTCATAACATCATAACATGACACTA TGAAGACACGATCTTCAGTAAACAG | 15729038–15729207 This study |
| KGC3_15.9    | INDEL F            | TCGGAGATATGCTATATAGGATGTA AATTTCACATCCAATACCTGGTCTGT | 15966551–15966800 This study |
| KGC3_16.1    | INDEL F            | GTTTAGATATGCCTCTGCGCAGCTGT CGTGTATAGGGTACGGCGC | 16117085–16117235 This study |
| KGC3_16.3    | INDEL F            | ATTAGATATCCACCAATAGGCgGCGC CAGGTCAGGATGAGCT | 16322999–16322546 This study |
| RM15189      | SSR F              | CAGTAAATGTGCTCGGAGACGCTG TGCGTACAGTTACCTTCTTCAGTAC | 16699297–16699465 IRGSP 2005, redesigned in this study |
| KGC3_16.7    | INDEL F            | TCGGAGATATGCTATATAGGATGTA TGAAGACACGATCTTCAGTAAACAG | 16726679–16726765 This study |
| RM15191      | SSR F              | CGTCAATACCATTTGGGCTTTTTGCTTTATATATATATCGT | 16747940–16748065 IRGSP 2005, redesigned in this study |
| RM15206      | SSR F              | GAAAGACTCAATAGTAGTACAAAGGAGA GAAAAGACTCAATAGTAGTACAAAGGAGA | 16965176–16965240 IRGSP 2005, redesigned in this study |
| KGC3_17.02   | INDEL F            | CGGAGAAATCTGATCGGAGG GGAAGACCTATGCA GCAAAATAGTGACAC | 17022626–17022820 This study |
| KGC3_17.03   | INDEL F            | GCCACACTGCTGACATT AGGTCCAAGGCAACGAC | 17034809–17034952 This study |
| KGC3_17.1    | INDEL F            | ACATGCTGCAATGGATGGGTGATCTTGCTTG CAGTGGTTCGCTTAGT | 17120606–17120778 This study |
| KGC3_17.2    | INDEL F            | GACGCCCACACACCATATGAC GAGGATGGGCGAAGGCTGCG | 17213199–17213308 This study |
| RM3400       | SSR F              | TCTCTCTCTCTCTCGTCTGGA TAAAGCCAAGATGGCTTCG | 17266171–17266354 McCouch et al. 2002 |
| RM7642       | SSR F              | ACAGGAAATACAGGGACCCTGG TTGAACATTTGCTGTAGGG | 18631946–18632139 McCouch et al. 2002 |
| RM16         | SSR F              | CGTAGGGGCGACATCTAAGAACAGGACAGG | 23127576–23127743 McCouch et al. 2002 |
Results

Genetic analysis

IR24 was found to be susceptible to the six Japanese Xoo races used for this study, whereas XM14 was resistant to them (Fig. 1, Table 2). The average LL of XM 14 reaction for the Japanese Xoo races was 0.4 cm, whereas that of IR24 reaction was 23.6 cm. The F2 population from the cross between IR24 and XM14 showed a clear bimodal distribution of LL using Xoo race IIA (T7147). We observed a clear gap and classified the 216 F2 plants into 53 resistant plants with LL of 0.1–2 cm and 163 susceptible plants with LL of 7–37 cm (Fig. 2). The ratio 53:163 fitted 1:3, one-gene segregation ($\chi^2 = 0.02$, $P = 0.88$). From the above result and subsequent linkage analysis, it seems readily apparent that XM14 carries a novel Xoo-resistance gene. Therefore, the gene identified in XM14 was named \textit{Xanthomonas oryzae pv. oryzae resistance 42} (\textit{xa42}) according to the gene nomenclature system for rice (McCouch and CGSNL 2008). \textit{Xa42} is a susceptible wild type allele, whereas \textit{xa42} is a resistant mutated allele.

Linkage analysis

The F2 population from the cross between Koshihikari and XM14 showed continuous distribution of LL using Xoo race II with no clear gap (Fig. 3), partly because Koshihikari (Japonica) and XM14 (Indica) have different backgrounds. Large variation in agronomic traits such as the tiller number and plant height caused by Indica–Japonica genetic difference might increase LL variation. Therefore, instead of normal linkage analysis, we adopted the analysis using extreme recessive phenotype proposed by Zhang \textit{et al.} (1994). Ten F2 plants with the shortest LL (0.1–4 cm) were selected, and DNA was extracted from each plant. Then genotyping was done using 113 SSR and Indel markers covering the whole rice genome (Table 3). If a DNA marker is linked closely to the resistance gene in XM14, then most or all of the ten resistant plants were homozygotes of XM14 allele at the DNA marker locus. Nine plants were homozygotes of XM14 allele at the consecutive four DNA marker loci, RM3400, RM6914, RM1334, and RM5684. Eight plants were homozygotes of XM14 allele at the neighboring DNA marker loci, RM6959, RM3204, RM7642, RM5488, RM411, RM3698, RM487, RM7395 and RM6832 on chromosome 3. These results strongly suggest that \textit{Xa42} is located on chromosome 3. Calculations of the recombination frequency based on extreme recessive phenotype proposed by Zhang \textit{et al.} (1994) (Table 3) also support that inference.

Table 2. Reactions in lesion length (cm) of XM14, IR24, Koshihikari, and IAS16 after inoculation of six Japanese races of \textit{Xanthomonas oryzae pv. oryzae}

| Rice accession | Xanthomonas oryzae pv. oryzae race | Mean | SD |
|---------------|----------------------------------|------|----|
|               | race I (strain T7174)            |      |    |
| XM14          | 0.3                              | 0.2  | 0.1 |
| IR24          | 30.9                             | 1.5  | 2.4 |
| Koshihikari   | 15.3                             | 5.6  | 2.4 |
| IAS16         | 13.6                             | 3.6  | 2.4 |
|               | race IIA (strain T7147)          |      |    |
| XM14          | 0.2                              | 0.1  | 0.1 |
| IR24          | 28.2                             | 2.2  | 2.4 |
| Koshihikari   | 21.3                             | 5.6  | 2.4 |
| IAS16         | 22.3                             | 3.6  | 1.1 |
|               | race IIIB (strain H9387)         |      |    |
| XM14          | 0.7                              | 0.2  | 0.1 |
| IR24          | 25.6                             | 3.8  | 2.4 |
| Koshihikari   | 20.6                             | 5.1  | 2.4 |
| IAS16         | 24.1                             | 3.1  | 1.1 |
|               | race III (strain T7133)          |      |    |
| XM14          | 1.2                              | 0.6  | 0.1 |
| IR24          | 20.6                             | 2.8  | 2.4 |
| Koshihikari   | 17.8                             | 1.2  | 2.4 |
| IAS16         | 20.6                             | 5.3  | 2.4 |
|               | race IV (strain H75373)          |      |    |
| XM14          | 0.3                              | 0.1  | 0.1 |
| IR24          | 28.7                             | 0.5  | 2.4 |
| Koshihikari   | 11.7                             | 2.4  | 2.4 |
| IAS16         | 21.9                             | 2.9  | 2.4 |
|               | race V (strain H75304)           |      |    |
| XM14          | 0.3                              | 0.1  | 0.1 |
| IR24          | 24.9                             | 3.9  | 2.4 |
| Koshihikari   | 22.7                             | 2.4  | 2.4 |
| IAS16         | 20.5                             | 5.1  | 2.4 |

\textsuperscript{a} Mean denotes the mean lesion length (cm) of three leaves 18 days after Xoo inoculation.

\textsuperscript{b} SD stands for standard deviation.
The two recombinant Plant Nos. 4 and 6 were homozygotes of XM14 allele because they showed LL shorter than 1.0 cm, and were fixed for resistant plants in F3 generation. In Plant No. 4, recombination event occurred between KGC3_16.1 and KGC3_16.3. In Plant No. 6, recombination event occurred between KGC3_16.3 and RM15189. XA42 is expected to be located near the loci at which genotypes of the recombinants were homozygotes of XM14 allele. Therefore, XA42 is located close to KGC3_16.3. Results for the other plants in Table 4 all support this idea. Therefore, the dividing point at 3.0 cm clearly classified the F2 plants into resistant homozygous plants of xA42 allele and susceptible plants with the other genotypes (Fig. 4).

Based on the classification, the linkage map surrounding XA42 is shown in Fig. 5. The linkage around XA42 locus was compared with a restriction fragment length polymorphism (RFLP) marker-based high-density linkage map (Harushima et al. 1998), in which some markers have been sequenced. Based on the Nipponbare genome sequence (Os-Nipponbare-Reference-IRGSP-1.0), DNA markers located near each other on Nipponbare pseudomolecules are connected with dotted lines (Fig. 5): XA42 is located around the centromeric region of rice chromosome 3.

Discussion

This study identified a new rice mutant line named XM14. Inoculation tests involving six Japanese Xoo races (I, IIA, IIB, III–V) were conducted, and XM14 exhibited resistance with the average LL of 0.4 cm across all races. Using F2 plants from the cross between XM14 mutant line and its original cultivar IR24 and inoculating Japanese Xoo race IIA (strain T7147), we confirmed that the resistance gene is controlled by a single recessive gene. Linkage analysis showed that this gene was located around the centromeric region of chromosome 3.

Currently about 40 genes conferring host resistance to Xoo have been identified and reported (http://www.shigen.nig.ac.jp/rice/oryzabase/locale/change?lang=en, Hutin et al. [2012]).
| Chromosome | DNA marker | Genotypea | Individualb | Recombination frequencyc |
|------------|------------|------------|-------------|--------------------------|
| 1          | RM1282     | H X H K K X X X X X X | 0.40 | |
|            | RM220      | X X H H H H H H X X X X | 0.55 | |
|            | RM259      | X H X H H H H X X X | 0.25 | |
|            | RM8132     | H H X H H H X X | 0.30 | |
|            | SI3623     | H K K K H H H H X X | 0.50 | |
|            | RM8129     | X K K K H H H H X X | 0.45 | |
|            | RM246      | X K K K K K K X X | 0.50 | |
|            | RM1297     | X H K K H H K H X | 0.55 | |
|            | RM212      | K X K K K K K H X | 0.60 | |
|            | RM5448     | K X X H H H H X X K | 0.55 | |
|            | RM8099     | K K H H H H H H H H H | 0.50 | |
| 2          | RM211      | H K K H H H H H H K | 0.65 | |
|            | RM5664     | H K K K X K H K K K | 0.70 | |
|            | RM6844     | H H H H H H H K K K | 0.70 | |
|            | RM29       | H H H H H H H H H X | 0.40 | |
|            | RM1303     | H H H H H H H H H H | 0.45 | |
|            | RM3525     | K K K H H H H H X H | 0.45 | |
|            | RM1367     | K X K K K K K K X X | 0.35 | |
|            | RM240      | H H K H X H H X X X | 0.35 | |
|            | RM6312     | H H K K H X X K X X | 0.35 | |
| 3          | RM322      | H H H H H H H H H K | 0.55 | |
|            | ES0581     | X H X X X X X X X H | 0.20 | |
|            | RM6959     | X X H X X X X X X X | 0.10 | |
|            | RM3204     | X X H X X X X X X X | 0.10 | |
|            | RM3400     | X X X X X X X X X X | 0.05 | |
|            | RM6914     | X X X X X X X X X X | 0.05 | |
|            | RM1334     | X X X X X X X X X X | 0.05 | |
|            | RM5684     | X X X X X X X X X X | 0.05 | |
|            | RM7642     | H X X X X X X X X X | 0.10 | |
|            | RM5488     | H X X X X X X X X X | 0.10 | |
|            | RM441      | H X X X X X X X X X | 0.10 | |
|            | RM3698     | H X X X X X X X X X | 0.10 | |
|            | RM3646     | H X X X X X X X X X | 0.10 | |
|            | RM487      | H X X X X X X X X X | 0.10 | |
|            | RM7395     | H X X X X X X X X X | 0.10 | |
|            | RM6832     | H X X X X X X X X X | 0.10 | |
|            | RM15451    | H X X X X X X X X X | 0.25 | |
|            | RM5532     | H X X X X X X X X X | 0.25 | |
|            | RM6266     | H X X X X X X X X X | 0.25 | |
|            | RM3513     | H X X X X X X X X X | 0.30 | |
|            | RM3436     | H X X X X X X X X X | 0.35 | |
|            | RM3525     | X X X X X X X X X | 0.30 | |
|            | RM3346     | H H H H H H H H X | 0.45 | |
|            | RM1221     | H H H H H H H H H H | 0.45 | |
| 4          | C61009     | X X X X X K K H K H X | 0.50 | |
|            | RM7279     | X X X X X K K H K H K | 0.40 | |
|            | RM6997     | X X X X X K H K K K | 0.45 | |
|            | RM303      | X H X X X K H K K K | 0.50 | |
|            | RM252      | H H H H H K K K X | 0.50 | |
|            | RM348      | H K K K K K K K X | 0.55 | |
|            | RM8217     | K H K H H H H H H | 0.60 | |
|            | RM6246     | K H K H H H H H X | 0.55 | |
| 5          | RM7373     | X X H H H H K X X | 0.40 | |
|            | RM3345     | X X H H H H K K X | 0.45 | |
|            | RM7444     | X X H K K K K X K | 0.55 | |
|            | RM3777     | K K K K K K K K X | 0.65 | |
|            | C50867     | K K K K K K K K K | 0.80 | |

| Chromosome | DNA marker | Genotypea | Individualb | Recombination frequencyc |
|------------|------------|------------|-------------|--------------------------|
| 1          | RM249      | H H K K K H X X X K | 0.40 | |
|            | RM7568     | H H H H X K K K X | 0.50 | |
|            | E60663     | H H K K X X H H K K | 0.60 | |
|            | RM6954     | H K K K H H H K K | 0.75 | |
|            | RM3476     | H K K K K K K K K | 0.70 | |
|            | E30287     | X X X H H H H H X | 0.20 | |
|            | RM2535     | X X X X X X X X X | 0.15 | |
|            | RM2576     | H X X X X H H X X | 0.50 | |
|            | RM527      | H X X X H H H X | 0.25 | |
|            | RM3628     | H H H X H H H X | 0.35 | |
|            | RM3628     | H H H X H H H X | 0.35 | |
|            | RM3672     | H H X X H H X K | 0.45 | |
|            | RM58114    | H K H H K H H H K | 0.60 | |
| 2          | RM211      | H K K H H H H H H K | 0.65 | |
|            | S20268     | H K K X X X K H H | 0.40 | |
|            | RM1134     | H K K X X H K H H | 0.45 | |
|            | RM3826     | H K H H X X H X X | 0.45 | |
|            | RM234      | X H H H X H X X X | 0.20 | |
|            | RM1412     | H X H H X X X X X | 0.10 | |
|            | RM1306     | X K K H H H H H H | 0.30 | |
| 3          | RM322      | H H H H H H H H H | 0.55 | |
|            | RM6369     | X H X H K K X X X K | 0.45 | |
|            | RM6771     | X H X H K H H X X | 0.35 | |
|            | RM6891     | X H H X X X X X X | 0.35 | |
|            | EG1552     | X H H X X X X K X | 0.30 | |
|            | RM257      | H X X H X X X X | 0.30 | |
|            | RM6971     | H K H X X X K K K | 0.45 | |
|            | E2191H     | H K H X X X K X X K | 0.45 | |
|            | RM216      | H K K X X X K H X X | 0.40 | |
|            | RM3795     | H K K X X X K K K | 0.45 | |
|            | RM258      | H H H X X X H X X | 0.50 | |
|            | RM1108     | H H K K H H H H X | 0.40 | |
|            | RM3525     | H K K K H H H H H | 0.55 | |
|            | RM228      | H K K H H H H H H | 0.55 | |
|            | RM4B       | X H H H H H X X X X | 0.40 | |

Table 3. Genotypes of 113 DNA markers covering the rice genomes of ten plants with the shortest lesion length derived from the F2 population from the cross between Koshihikari and XM14.

a X, H, and K respectively denote homozygotes for XM14, heterozygotes, and homozygotes for Koshihikari.
b Ten plants with the shortest lesion length (0.1–4 cm) 18 days after inoculation with Xoo race IIA (strain T7147) were selected.
c DNA makers are arranged based on the physical distance from the end of short arm of each chromosome.
d Recombination frequency is calculated as (N1 + N2)/N, in which N is the total number of plants surveyed (in this case, ten), N1 is the number of homozygotes of Koshihikari, and N2 is the number of heterozygotes (Zhang et al. 1994).
Among all resistance genes, only Xa11 has been reported to be located on chromosome 3, not around the centromeric region but on the long arm (Goto et al. 2009). Located around the centromeric region of chromosome 3, a new gene name xa42 was assigned to this resistant gene in XM14, according to the gene nomenclature system for rice (McCouch and CGSNL 2008). Many resistance genes ‘break down’ when they have been widely used for many years in a large population. Exploitation of new resistance genes is urgently necessary. The new resistant gene xa42 in this study is expected to be useful in resistance breeding programs and genetic analysis of Xoo resistance.

To the six Japanese Xoo races used for this study, XM14 is resistant. xa42 gene in XM14 has been proven to confer resistance against Japanese race IIA (strain T7147). We are not sure that xa42 is resistant to Xoo races other than Japanese race IIA because it is difficult to inoculate plants in segregating populations with many races. Because the probability of identifying Xoo resistant mutant is small (Taura et al. 1991a), the existence of simultaneous plural resistance mutations on one M2 line seems improbable. Therefore, it is plausible that xa42 confers resistance to all races with which XM14 was inoculated. The fact that many of resistant genes reported to date have shown resistance to plural races supports this idea. Future studies using the progeny of

---

**Table 4.** Genotypes of informative recombinants and non-recombinants for the DNA marker loci linked with XA42 on chromosome 3 in the F2 population (XM14 × IAS16), and the gene segregation in the F3 generation

| F2 Individual | Lesion length b (cm) | Reaction c | Genotypes of the DNA marker loci a | No. of F3 plants | Reaction d |
|---------------|---------------------|------------|----------------------------------|----------------|------------|
|               |                     |            | KGC<sub>3</sub> 15.6 | KGC<sub>3</sub> 16.1 | KGC<sub>3</sub> 16.3 | RM 15158 | RM 15191 | RM 15206 | RM 17.02 | RM 17.03 | RM 17.1 | RM 7,642 | RM 16 | |
| 1             | 57.3                | S          | H H H H H H H X X X X X X X | 7 23 |
| 2             | 20.6                | S          | H H H H H H H X X X X X X | 9 21 |
| 3             | 19.6                | S          | H H H H H H H X X X X X X | 8 22 |
| 4             | 0.4                 | R          | R X X X X X X X X X X X X | 17 0 |
| 5             | 0.6                 | R          | R X X X X X X X X X X X X | NT NT |
| 6             | 0.9                 | R          | R X X X X X X X X X X X X | 30 0 |
| 7             | 21.2                | S          | S H H H H H H H H H H H H | 5 11 |
| 8             | 24.6                | S          | S A A A A A A A H H H H H H | 0 18 |
| 9             | 31.5                | S          | S H H A A A A A A A A A A A | 0 11 |
| 10            | 30.1                | S          | S H H H H H H H A A A A A A | 5 17 |
| 11            | 29.6                | S          | S H H H H H H H A A A A A A | 3 17 |
| 12            | 0.1                 | R          | R X X X X X X X X X X X X | NT NT |
| 13            | 2.8                 | R          | R X X X X X X X X X X X X | 15 0 |
| 14            | 4.5                 | R          | R A A A A A A A A A A A A | 0 15 |
| 15            | 8.6                 | R          | R A A A A A A A A A A A A | NT NT |
| 16            | 9.3                 | R          | R A A A A A A A A A A A A | NT NT |
| 17            | 21.2                | S          | S H H H H H H H H H H H H | NT NT |
| 18            | 21.4                | S          | S A A A A A A A A A A A A | NT NT |
| 19            | 32.1                | S          | S A A A A A A A A A A A A | NT NT |
| 20            | 32.3                | S          | S A A A A A A A A A A A A | NT NT |

a X, H, and A respectively denote homozygotes for XM14, heterozygotes, and homozygotes for IAS16.
b Lesion lengths were recorded 18 days after inoculation with Xoo race IIA (strain T7147).
c R and S respectively denote resistant and susceptible. Plants with lesion length of 0.1–2.8 cm were regarded as R. Those with lesion length of 4.5–60.1 cm were regarded as S.
d Scoring of LL of F3 lines from the cross between XM14 and IAS16 was based on visual observations: LL shorter than 3 cm as judged by visual observation were scored as R, whereas those longer than 3 cm were scored as S.
e Not tested.
Identification and linkage analysis of rice bacterial blight resistance gene

...located near each other on Nipponbare pseudomolecules are connected by dotted lines.

...a combination of plural genes. To confirm that it is a truly a gene only or by... 

...XA42 gene on chromosome was transmitted more than... 

...Spectra of isolated recessive resistance genes to Xoo race are also different: The homozygotes of xa5 is resistant to Japanese races IA, IB, IIIA, IIIB, and IV, Philippine races 1–5, susceptible to Philippine race 6 (Ogawa et al. 1991). The homozygotes of xa13 are susceptible to Philippine races 1–5 (Singh et al. 2001), and resistant to Philippine race 6 (Chu et al. 2006), which is virulent to most resistant genes (Ogawa et al. 1991). The homozygotes of xa25 are susceptible to... 

...Xoo symptoms can be affected by environmental conditions and the rice developmental stage (Mew 1987). The genetic background of IR24 in XM14 has a great benefit for those studying Xoo resistance in rice. Experimental lines of many kinds for Xoo resistance have been constructed under IR24 genetic background: near-isogenic lines carrying single Xoo resistance gene (Ogawa et al. 1991), pyramid lines carrying multiple resistance genes (Huang et al. 1997, Yoshimura et al. 1996), and artificially induced mutant lines (Taura et al. 1991a). Therefore, the effect of newly identified genes such as xa42 on resistance to Xoo can be compared easily with other previously described genes. The effect of pyramiding of xa42 with other genes can also be evaluated easily. Iyer-Pascuzzi and McCouch (2007) reviewed the genetic and molecular resistance mechanism of xa5 and xa13 with special emphasis on their recessive inheritance. Regarding pyramiding, when used in combination with other resistance genes, both xa5 and xa13 provide stronger and broader levels of resistance than when used alone. We are undertaking the pyramiding of xa42 with other resistance genes. In this study, the combination of rough linkage analysis using extreme recessive phenotype using Indica–Japonica cross and precise linkage analysis using CSSLs effectively mapped recessive mutant resistance gene induced in IR24. The same mapping strategy can be applied to other previously identified Xoo resistant mutants with IR24 background such as XM5 (Taura et al. 1991b) and XM6 (Taura et al. 1992), which will also contribute to the study of Xoo resistance in rice.

...Selection of Xoo resistant recessive mutant plants in padd...
Acknowledgements

The authors gratefully acknowledge Dr. Atsushi Yoshimura of Kyushu University for the kind provision of IAS lines. The authors are also grateful to Dr. Toyoaki Anai of Saga University for reading the manuscript.

Literature Cited

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

Chu, Z., B. Fu, H. Yang, C. Xu, Z. Li, A. Sanchez, Y.J. Park, J.L. Bennetzen, Q. Zhang and S. Wang (2006) Targeting xa13, a recessive gene for bacterial blight resistance in rice. Theor. Appl. Genet. 112: 455–461.

Bellporta, S.L., J. Wood and J.B. Hicks (1983) A plant DNA minipreparation: version II. Plant Mol. Biol. Rep. 1: 19–21.

Fukuta, Y., H. Sasahara, K. Tamura and T. Fukuyama (2000) RFLP linkage map included the information of segregation distortion in a wide-cross population between Indica and Japonica rice (Oryza sativa L.). Breed. Sci. 50: 65–72.

Gao, Z.Y., S.C. Zhao, W.M. He, L.B. Guo, Y.L. Peng, J.J. Wang, X.S. Guo, X.M. Zhang, Y.C. Rao, C. Zhang et al. (2013) Dissecting yield-associated loci in super hybrid rice by resequencing recombinant inbred lines and improving parental genome sequences. Proc. Natl. Acad. Sci. USA 110: 14492–14497.

Gonzalez, C., B. Szurek, C. Manceau, T. Mathieu, Y. Sere and V. Verdière (2007) Molecular and pathotypic characterization of new Xanthomonas oryzae strains from West Africa. Mol. Plant Microbe Interact. 20: 534–546.

Goto, T., T. Matsumoto, N. Furuya, K. Tsuchiya and A. Yoshimura (2009) Mapping of bacterial blight resistance gene Xa11 on rice chromosome 3. JARQ 43: 221–225.

Harashima, Y., M. Yano, A. Shomura, M. Sato, T. Shimano, Y. Kuboki, T. Yamamoto, S.Y. Lin, B.A. Antonio, A. Parco et al. (1998) A high-density rice genetic linkage map with 2275 markers using a single F2 population. Genetics 148: 479–494.

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

Hutin, M., F. Sabot, A. Ghesquiêre, R. Koebnik and B. Szurek (2015) A knowledge-based molecular screen uncovers a broad-spectrum OsSWEET14 resistance allele to bacterial blight from wild rice. Plant J. 84: 694–703.

Ichitani, K., D. Yamaguchi, S. Taura, Y. Fukutoku, M. Onoue, K. Shimizu, F. Hashimoto, Y. Sakata and M. Sato (2014) Genetic analysis of ion-beam induced extremely late heading mutants in rice. Breed. Sci. 64: 222–230.

International Rice Genome Sequencing Project (IRGSP) (2005) The map-based sequence of the rice genome. Nature 436: 793–800.

Iwata, H. and S. Ninomiya (2006) AntMap: constructing genetic linkage maps using an ant colony optimization algorithm. Breed. Sci. 56: 371–377.

Iyer, A.S. and S.R. McCouch (2004) The rice bacterial blight resistance gene xa5 encodes a novel form of disease resistance. Mol. Plant Microbe Interact. 17: 1348–1354.

Iyer-pasuzzi, A.S. and S.R. McCouch (2007) Recessive resistance genes and the Oryza sativa–Xanthomonas oryzae pv. oryzae pathosystem. Mol. Plant Microbe Interact. 20: 731–739.

Kaku, H. and T. Kimura (1989) Qualitative resistance reaction of rice cultivar Asominori to certain race II strains of Xanthomonas campestris pv. oryzae. Ann. Phytopath. Soc. Japan 55: 657–659.

Kauffman, E., A.P.K. Reddy, S.P.Y. Hsien and S.D. Merca (1973) An improved technique for resistance of rice varieties to Xanthomonas oryzae. Plant Dis. Rep. 57: 537–541.

Kawahara, Y., M. Bastide, J.P. Hamilton, H. Kanamori, W.R. McCombie, S. Ouyang, D.C. Schwartz, T. Tanaka, J. Wu, S. Zhou et al. (2013) Improvement of the Oryza sativa Nipponbare reference genome using next generation sequence and optical map data. Rice 6: 4.

Khan, A.M., M. Naeem and M. Iqbal (2014) Breeding approaches for bacterial leaf blight resistance in rice (Oryza sativa L.), current status and future directions. Eur. J. Plant Pathol. 139: 27–37.

Khush, G.S. (2005) What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol. Biol. 59: 1–6.

Kim, S.M., J.P. Suh, Y. Qin, T.H. Noh, R.F. Reinke and K.K. Jena (2015) Identification and fine-mapping of a new resistance gene, Xa40, conferring resistance to bacterial blight races in rice (Oryza sativa L.). Theor. Appl. Genet. 128: 1933–1943.

Kosambi, D.D. (1944) The estimation of map distances from recombination values. Ann. Eugen. 12: 172–175.

Kubo, T., Y. Aida, K. Nakamura, H. Tsunematsu, K. Dori and A. Yoshimura (2002) Reciprocal chromosome segment substitution series derived from Japonica and Indica cross of rice (Oryza sativa L.). Breed. Sci. 52: 319–325.

Liu, Q., M. Yuan, Y. Zhou, X. Li, J. Xiao and S. Wang (2011) A paralog of the MtN3/saliva family secretes race-specific resistance to Xanthomonas oryzae in rice. Plant Cell Environ. 34: 1958–1969.

McCouch, S.R., L. Teytelman, Y. Xu, K.B. Lobos, K. Clare, M. Walton, B. Fu, R. Maghirang, Z. Li, Y. Xing et al. (2002) Development and mapping of 2240 new SSR markers for rice (Oryza sativa L.). DNA Res. 9: 199–207.

McCouch, S.R. and Committee on Gene Symbolization, Nomenclature and Linkage, Rice Genetics Cooperative (CGSNL) (2008) Gene nomenclature system for rice. Rice 1: 72–84.

Mew, T.W. (1987) Current status and future prospects of research on bacterial blight of rice. Annu. Rev. Phytopathol. 25: 359–382.

Mew, T.W., A.M. Alvarez, J.E. Leach and J. Swings (1993) Focus on bacterial blight of rice. Plant Dis. 77: 5–12.

Mishra, D., M.R. Vishnupriya, M.G. Anil, K. Konda, Y. Raj and V. Sonti (2013) Pathotype and genetic diversity amongst Indian isolates of Xanthomonas oryzae pv. oryzae. PLoS ONE 8: e81996.

Niño-Liu, D.O., P.C. Ronald and A.J. Bogdanove (2006) Xanthomonas oryzae pv. oryzae pathogens: model pathogens of a model crop. Mol. Plant Pathol. 7: 303–324.

Noda, T. and A. Ohuchi (1989) A new pathogenic race of Xanthomonas campestris pv. oryzae and inheritance of resistance of differential rice variety, Te-tep to it. Ann. Phytopath. Soc. Japan 55: 201–207.

Ogawa, T. and T. Yamamoto (1987) Reaction of rice cultivars resistant to Japanese and Philippine races of Xanthomonas campestris pv. oryzae. JARQ 21: 138–145.

Ogawa, T. and G.S. Khush (1989) Major genes for resistance to bacterial blight in rice. In: Bacterial blight of rice, International Rice Research Institute, Manila, pp. 178–192.

Ogawa, T., T. Yamamoto, G.S. Khush and T.W. Mew (1991) Breeding of near-isogenic lines of rice with single genes for resistance to bacterial blight pathogen (Xanthomonas campestris pv. oryzae).
Identification and linkage analysis of rice bacterial blight resistance gene

Japan. J. Breed. 41: 523–529.

Ou, S.H. (1985) Rice diseases, second edition. Commonwealth Mycological Institute, Kew, p. 380.

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

Sakai, H., S.S. Lee, T. Tanaka, H. Numa, J. Kim, Y. Kawahara, H. Wakimoto, C.C. Yang, M. Iwamoto, T. Abe et al. (2013) Rice Annotation Project Database (RAP-DB): an integrative and interactive database for rice genomics. Plant Cell Physiol. 54: e6.

Singh, S., J.S. Sidhu, N. Huang, Y. Vikal, Z. Li, D.S. Brar, H.S. Dhaliwal and G.S. Khush (2001) Pyramiding three bacterial blight resistance genes (xa3, xa13 and Xa21) using marker-assisted selection into indica rice cultivar PR106. Theor. Appl. Genet. 102: 1011–1015.

Suzuki, T., M. Eiguchi, T. Kumamaru, H. Satoh, H. Matsusaka, K. Moriguchi, Y. Nagato and N. Kurata (2008) MNU-induced mutant pools and high performance TILLING enable finding of any gene mutation in rice. Mol. Genet. Genomics 279: 213–223.

Taura, S., T. Ogawa, A. Yoshimura and T. Omura (1991a) Induction of mutants resistant to bacterial blight in rice. Japan. J. Breed. 41: 279–288.

Taura, S., T. Ogawa, A. Yoshimura, R. Ikeda and T. Omura (1991b) Identification of a recessive resistance gene in induced mutant line XM5 of rice to rice bacterial blight. Japan. J. Breed. 41: 427–432.

Taura, S., T. Ogawa, A. Yoshimura, R. Ikeda and N. Iwata (1992) Identification of a recessive resistance gene to rice bacterial blight of mutant line XM6, Oryzae sativa L. Japan. J. Breed. 42: 7–13.

Till, B.J., J. Cooper, T.H. Tai, P. Colowit, E.A. Greene, S. Henikoff and L. Comai (2007) Discovery of chemically induced mutations in rice by TILLING. BMC Plant Biol. 7: 19.

Untergrasser, A., I. Cutcutache, T. Koresaar, J. Ye, B.C. Faircloth, M. Remm and S.G. Rozen (2012) Primer3—new capabilities and interfaces. Nucleic Acids Res. 40: e115.

Verdier, V., C. Vera Cruz and J.E. Leach (2012) Controlling rice bacterial blight in Africa: needs and prospects. J. Biotechnol. 159: 320–328.

Wakimoto, S. (1954) Biological and physiological properties of Xanthomonas oryzae phage. Sci. Bull. Fac. Agric, Kyushu Univ. 14: 485–493.

Xia, C., H. Chen and H. Zhu (2012) Identification, mapping, isolation of the genes resisting to bacterial blight and breeding application in rice. Mol. Plant Breed. 3: 121–131.

Xu, X., X. Liu, S. Ge, J.D. Jensen, F. Hu, X. Li, Y. Dong, R.N. Gutenkunst, L. Fang, L. Huang et al. (2012) Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. Nat. Biotechnol. 30: 105–111.

Yamazaki, Y., S. Sakaniwa, R. Tsuchiya, K.I. Nonomura and N. Kurata (2010) Oryzabase: an integrated information resource for rice science. Breed. Sci. 60: 544–548.

Yang, B., A. Sugio and F.F. White (2006) Os8N3 is a host disease-susceptibility gene for bacterial blight of rice. Proc. Natl. Acad. Sci. USA 103: 10503–10508.

Yoshimura, A., J.X. Lei, T. Matsumoto, H. Tsunematsu, S. Yoshimura, N. Iwata, M.R. Baraoidan, T.W. Mew and R.J. Nelson (1996) Analysis of pyramiding of bacterial blight resistance genes in rice by using DNA markers. In: Khush, G.S. (ed.) Rice Genetics III, International Rice Research Institute, Manila, pp. 577–581.

Yu, J., S. Hu, J. Wang, G.K. Wong, S. Li, B. Liu, Y. Deng, L. Dai, Y. Zhou, X. Zhang et al. (2002) A draft sequence of the rice genome (Oryza sativa L. spp. indica). Science 296: 79–92.

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