Confirming a major QTL and finding additional loci responsible for field resistance to brown spot (Bipolaris oryzae) in rice

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Brown spot is a devastating rice disease. Quantitative resistance has been observed in local varieties (e.g., ‘Tadukan’), but no economically useful resistant variety has been bred. Using quantitative trait locus (QTL) analysis of recombinant inbred lines (RILs) from ‘Tadukan’ (resistant) × ‘Hinohikari’ (susceptible), we previously found three QTLs (qBS2, qBS9, and qBS11) that conferred resistance in seedlings in a greenhouse. To confirm their effect, the parents and later generations of RILs were transplanted into paddy fields where brown spot severely occurred. Three new resistance QTLs (qBSfR1, qBSfR4, and qBSfR11) were detected on chromosomes 1, 4, and 11, respectively. The ‘Tadukan’ alleles at qBSfR1 and qBSfR11 and the ‘Hinohikari’ allele at qBSfR4 increased resistance. The major QTL qBSfR11 coincided with qBS11 from the previous study, whereas qBSfR1 and qBSfR4 were new but neither qBS2 nor qBS9 were detected. To verify the qBSfR1 and qBSfR11 ‘Tadukan’ resistance alleles, near-isogenic lines (NILs) with one or both QTLs in a susceptible background (‘Koshihikari’) were evaluated under field conditions. NILs with qBSfR11 acquired significant field resistance; those with qBSfR1 did not. This confirms the effectiveness of qBSfR11. Genetic markers flanking qBSfR11 will be powerful tools for marker-assisted selection to improve brown spot resistance.

Key Words: Bipolaris oryzae, brown spot, Oryza sativa L., QTL analysis, field resistance, rice.
previously identified three quantitative trait loci (QTLs) that contributed to brown spot resistance at the seedling stage using recombinant inbred lines (RILs) from a cross of ‘Tadukan’ with ‘Hinohikari’ (Sato et al. 2008a). The objectives of this study were to confirm the effects of these resistance QTLs under field conditions and to introduce them into a regionally adapted variety, such as ‘Koshihikari’. Based on the results of our field trials, we discuss the usefulness of marker-assisted selection (MAS) to improve brown spot field resistance in rice.

**Materials and Methods**

**Fungal isolation and inoculation**

The Iga-2 strain of *Bipolaris oryzae* was isolated from rice leaves collected in a research field at the Mie Prefecture Agricultural Research Institute (MPARI, Iga, Mie, Japan) using the technique described by Kihara and Kumagai (1994). The stock culture is maintained in MPARI. Inoculation, culture of the mycelia, and induction of conidiophore formation under irradiation with black light lamps were also based on the methods of Kihara and Kumagai (1994).

**Plant materials**

We used 110 RILs in the F\textsubscript{8} generation derived from crosses between ‘Tadukan’ (resistant) and ‘Hinohikari’ (susceptible) in the present resistance testing and QTL mapping for field resistance to brown spot. The RILs were advanced from different F\textsubscript{2} plants by means of single-seed descent; an intermediate generation (F\textsubscript{3}) had been previously used to identify three QTLs for brown spot resistance at the seedling stage (Sato et al. 2008a). On the basis of our initial field resistance and mapping results, we selected one RIL (THRIL50) that possessed putative QTLs for field resistance to brown spot, and crossed it with a susceptible variety, ‘Koshihikari’. One F\textsubscript{1} plant was backcrossed three times with ‘Koshihikari’ to introduce the QTLs into the ‘Koshihikari’ background. We obtained 19 near-isogenic lines (NILs) in the BC\textsubscript{1}F\textsubscript{3} generation, which were grown to evaluate their field resistance.

**Field evaluation of brown spot resistance**

In 2012 and 2013, brown spot resistance was evaluated for QTL mapping in a paddy field at MPARI, with two replications, following the procedure of Matsumoto et al. (2014). To initiate the disease in the field, spreader plants (cv. ‘Mienoyume’, susceptible) that had been inoculated with the Iga-2 strain were planted at a rate of three to five plants per hill, at a spacing of 30 × 30 cm among the plants. Then, each RIL and its parents were transplanted (11 hills, 30 × 15 cm) between the spreader rows. Disease scores were recorded 113 days after transplanting, using a scale from 0 to 9 (Supplemental Table 1). In 2013, the brown spot field resistance of the 19 NILs was examined using the same method.

**DNA marker assays**

Total DNA was extracted from the leaves using the CTAB method (Murray and Thompson 1980). To construct a linkage map, we used 169 polymorphic markers: 162 rice simple-sequence repeat (SSR) markers and 7 sequence-tagged site (STS) markers. SSR and STS analysis were carried out according to the protocol of Sato et al. (2008a). Linkage groups and the order of the markers were determined using version 3 of the MAPMAKER/EXP software (Lander et al. 1987). The resulting genetic linkage map was visualized by using a Microsoft Excel macro, MapDraw (Liu and Meng 2003). QTL analysis was performed using version 2.5 of Windows QTL cartographer (Wang et al. 2006) with the default composite interval mapping and control parameters, model 6 of the standard model, five control markers, a 10-cM window size, and the forward and backward regression model. We used genome-wide threshold values (α = 0.05) to detect putative QTLs on the basis of the results of 1000 permutations. To survey the genotypes of NILs that harbored putative brown spot resistance QTLs, single-nucleotide polymorphism (SNP) analysis was employed. A 384-plex set of SNP markers was selected from diverse accessions of cultivated Asian rice (Ebana et al. 2010). Genotyping was performed by using the GoldenGate BeadArray technology platform (Illumina Inc., San Diego, CA, USA). These SNPs were detected by using the Illumina Bead Station 500G system. All experimental procedures for the SNP typing followed the manufacturer’s instructions.

**Results**

**Phenotypic analysis of parental lines and their progeny**

Distinct differences in brown spot field resistance were observed between the parental varieties, ‘Tadukan’ and ‘Hinohikari’: the mean disease scores in 2012 and 2013 were 2.2 ± 0.5 and 5.3 ± 0.8, respectively (Fig. 1, 2). The disease score ratings of the RIL populations in 2012 and 2013 were normally distributed, and a certain number of
lines exhibited transgressive segregation in both directions, which indicated that the inheritance of field resistance was quantitative (Fig. 2).

QTLs for field resistance

The 162 SSR and 7 STS polymorphic markers were used for map construction. This map covered a total genetic distance of 1286.4 cM and provided partial linkage groups for all chromosomes, except for small gaps in chromosomes 4, 6, and 8 (Data not shown). Three QTLs for field resistance (qBSfR1, qBSfR4, and qBSfR11) were identified based on the data from 2012 and 2013 (Table 1). These QTLs were located on chromosomes 1, 4, and 11. The ‘Tadukan’ alleles of qBSfR1 and qBSfR11 explained 10.4 to 12.3% and 17.9 to 19.2% of the total phenotypic variation, respectively; qBSfR4 from ‘Hinohikari’ accounted for 10.2 to 10.8% of the total variation. The position of the major QTL qBSfR11 coincided with that of the resistance allele qBS11 that was detected in ‘Tadukan’ at the seedling stage (Fig. 3; Sato et al. 2008a). However, the two other QTLs differed from the resistance alleles qBS2 and qBS9 reported by Sato et al. (2008a) (Fig. 3). Thus, in spite of the difference in growth stages analyzed in the two studies, a major resistance QTL from ‘Tadukan’ was detected in both experiments, whereas the relatively minor QTLs detected in the present study did not correspond to those in the previous study (Sato et al. 2008a).

Verification of QTLs for field resistance

To verify the effects of the ‘Tadukan’ resistance QTLs, 19 NILs from the BC3F5 generation, which were derived from the same BC3F1 plant, were genotyped using SNP markers and their disease phenotypes were evaluated in a field test (Table 2). ‘Koshihikari’, the susceptible recurrent parent of the NILs, is a parent of ‘Hinohikari’ (‘Aichi 40’/‘Koshihikari’); there were no markers around the qBSfR4 region that were polymorphic between ‘Koshihikari’ and ‘Hinohikari’ (data not shown). Since all of the 19 lines possessed the same allele as ‘Koshihikari’ at the qBSfR4 locus, we have not further explored the effectiveness of qBSfR4 itself.

The mean disease scores of ‘Tadukan’ and ‘Koshihikari’ were 2.5 and 6.0, respectively. Two lines that possessed the ‘Tadukan’ allele of qBSfR1 showed slightly lower disease scores (i.e., slightly better resistance) than ‘Koshihikari’, with values of 5.0 for R307-240-4 and 5.5 for R307-147-2. However, the difference was not significant for either line. Among the 12 lines that possessed the major resistance QTL from ‘Koshihikari’ (qBSfR11), 11 lines had significantly lower disease scores than ‘Koshihikari’ (3.0 to 4.0) and were considered resistant; R307-89-9, with a disease score of 5.5, was considered to be as susceptible as ‘Koshihikari’. From this comparison of the phenotypes and graphical genotypes, it seems likely that qBSfR11 lies around the SNP marker interval; AE11005627-AE11000941. Although 5 lines (R225-1, -2, -3, and -4, and R306-1) harbored both of the ‘Tadukan’ resistance QTLs (qBSfR1 and qBSfR11), they showed disease scores (3.5 to 4.0) that were not significantly different from the 11 lines that possessed only qBSfR11 from ‘Tadukan’ (3.0 to 4.0), so no practical gene pyramiding effect was observed in our experiment.

Discussion

Many agriculturally important rice varieties are susceptible to brown spot, and show yield losses of 16 to 43% in inoculated plots (Datnoff et al. 1991, Yamaguchi et al. 1992). No major genes conferring immunity to brown spot have been identified, possibly because of the absence of physiological races of the fungus (Eruotor 1985, Sreegharan and Menon 1974). However, a few resistant varieties, such as ‘Tadukan’, offer sufficiently high quantitative resistance that they will be agriculturally useful (Eruotor 1985, Ohata and Kubo

Table 1. Putative QTLs for field resistance to brown spot of rice detected by using RILs (Tadukan/Hinohikari)

| Years | QTL     | Chromosome | Peak position (cM) | Nearest marker | LOD score | Variance explained of total (%) | Additive effect* |
|-------|---------|------------|--------------------|---------------|-----------|---------------------------------|-----------------|
| 2012  | qBSfR1  | 1          | 40.7               | RM10604       | 4.8       | 10.4                            | -0.3            |
|       | qBSfR4  | 4          | 91.4               | RM273         | 4.3       | 10.2                            | 0.3             |
|       | qBSfR11 | 11         | 52.4               | RM26992       | 8.0       | 17.9                            | -0.4            |
| 2013  | qBSfR1  | 1          | 44.7               | RM10604       | 4.9       | 12.3                            | -0.4            |
|       | qBSfR4  | 4          | 92.4               | RM273         | 4.8       | 10.8                            | 0.3             |
|       | qBSfR11 | 11         | 56.6               | RM27096       | 8.6       | 19.2                            | -0.5            |

* Negative values mean that the ‘Tadukan’ allele decreases the disease score (i.e., improves field resistance).
QTLs for rice brown spot resistance

Breeding Science
Vol. 65 No. 2

In the present study, we aimed to confirm the location and effect of these previously reported resistance QTLs and identify additional QTLs for resistance under field conditions. By using the newly constructed partial linkage map, we were able to identify three QTLs (qBSfR1, qBSfR4, and qBSfR11) for field resistance in the RILs (Fig. 3, Table 1). The ‘Tadukan’ alleles at the qBSfR1 and qBSfR11 loci significantly decreased the disease score (Table 1). Previously reported that three QTLs (qBS2, qBS9, and qBS11) contributed to resistance at the seedling stage and that ‘Tadukan’ provided the resistance alleles for qBS9 and qBS11 (Sato et al. 2008a). Of these QTLs, only the major QTL on chromosome 11 was stably found in both the greenhouse and field studies, whereas the minor loci were detected in only one of the two environments. This discrepancy could be due to differences in the growth stage or growth conditions. Similarly, the indica rice variety ‘Kasalath’ showed moderate resistance to brown spot at the seedling stage in a greenhouse, and genetic analysis revealed a gene on chromosome 9 that conferred moderate resistance (Sato et al. 2008b). However, the resistance during early vegetative stages was not found in an infected field (Matsumoto et al. 2014). Thus, to confirm the effect of resistance QTLs in a rice breeding program, it would be necessary to conduct field trials to confirm greenhouse results.

We created 19 NILs that contained the two resistance QTLs (qBSfR1 and qBSfR11) from ‘Tadukan’ in the susceptible variety ‘Koshihikari’, and evaluated their phenotypes in a field test (Table 2). Since the 11 NILs that possessed the qBSfR11 allele region from ‘Tadukan’ showed significantly higher field resistance than the parent (Table 2), this QTL appears likely to be agronomically useful. Based on the genotype of the “susceptible” line that harbors the ‘Tadukan’ qBSfR11 region, R307-89-9, it is likely that qBSfR11 lies around the SNP marker interval; AE11005627–AE11000941 (Table 2). In future work, this genetic information will facilitate the precise mapping and cloning of one or more resistance genes in the region. On the other hand, as none of the NILs that possessed the ‘Tadukan’ qBSfR1 allele showed significantly improved disease scores (Table 2), qBSfR1 is likely to be a false field resistance locus.
Table 2. Genotypes of 13 SNP markers and disease scores for brown spot of rice in the BC$_3$F$_5$ lines (Koshihikari*3//Tadukan/Hinohikari)

| Lines          | Genotype of SNP Marker in the BC$_3$F$_5$ lines$^a$ | Disease score$^c$ |
|---------------|---------------------------------------------------|-------------------|
|               | qBSR 1 region                                      |                   |
|               | AE01003285 (7.8)$^b$ | A                  | 2.5 ± 0.7 **    |
|               | P0634_1 (9.5)$^b$      | A                  | 6.0 ± 0.0      |
|               | ad01004243 (10.0)$^b$ | A                  | 5.0 ± 0.0      |
|               | ah01000747 (10.7)$^b$ | A                  | 5.5 ± 0.7      |
| Tadukan       | qBSR 4 region                                      |                   |
|               | P0709_2 (19.7)$^b$      | A                  | 4.0 ± 0.0 *    |
|               | ad04008446 (22.2)$^b$ | B                  | 3.5 ± 0.7 **   |
|               | ad04008616 (23.5)$^b$ | B                  | 3.5 ± 0.7 **   |
|               | ad04009558 (25.8)$^b$ | B                  | 3.5 ± 0.7 **   |
| Koshihikari   | qBSR 11 region                                     |                   |
|               | AE11002117 (17.9)$^b$ | A                  | 3.0 ± 0.0 **   |
|               | ah11000824 (21.0)$^b$ | B                  | 3.5 ± 0.7 **   |
|               | P0511 (22.9)$^b$      | B                  | 3.5 ± 0.7 **   |
|               | AE11005627 (25.3)$^b$ | B                  | 3.5 ± 0.7 **   |
|               | ah11000941 (25.6)$^b$ | B                  | 3.5 ± 0.7 **   |
| R307-240-4    | A                                                  | A                  | 5.5 ± 0.7      |
| R307-147-2    | A                                                  | A                  | 5.5 ± 0.7      |

$^a$ Genotypes of the SNP markers are represented by A (gray shading) for homozygous ‘Tadukan’ and B (white) for homozygotes ‘Koshihikari’.

$^b$ Numbers in parentheses beside the SNP markers indicate their physical map position (Mbp) in the chromosomes, according to the number of International Rice Genome Sequence Project (IRGSP) ver.1.0.

$^c$ Disease scores for brown spot are showed as Mean ± SD. *, ** indicate a significant difference from ‘Koshihikari’ (Dunnet’s test, P < 0.05 and P < 0.01, respectively).
Katara et al. (2010) recently identified 10 QTLs for field resistance in double-haploid lines derived from CT9993/IR62266, and 3 of these QTLs (on chromosomes 2, 9, and 11) were located in similar marker intervals to those of the QTLs in our previous and present studies \((qBS2, qBS9, qBS11, \text{and} qBSIR11)\). In addition, \(qBSIR4\) from the present study was near the \(Bsqd4.1\) locus mapped by Katara et al. (2010). This work in India confirms that QTL analysis for brown spot resistance can provide an effective framework for MAS in a rice breeding program. The QTL \(qBSIR11\) whose existence and location we confirmed in the present study could be useful in MAS-supported breeding to enhance resistance to brown spot disease. Now we promote strongly the MAS in our breeding programs to breed the practical varieties provided with resistance in near future.

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