Introduction

Rice brown spot (BS), caused by Bipolaris oryzae, is one of the major diseases of rice in Japan. Quantitative resistance has been observed in local cultivars (e.g., CH45), but no economically useful resistant variety has been bred. Using simple sequence repeat (SSR) polymorphic markers, we conducted quantitative trait locus (QTL) analysis of BS resistance in backcross inbred lines (BILs) from a cross between indica CH45 (resistant) and japonica Koshihikari (susceptible). On the basis of field disease evaluations in 2015 and 2016, four QTLs contributing to BS resistance were identified on chromosomes 2 (qBSR2-kc), 7 (qBSR7-kc), 9 (qBSR9-kc), and 11 (qBSR11-kc). The ‘CH45’ alleles at qBSR2-kc, qBSR7-kc, and qBSR11-kc and the ‘Koshihikari’ allele at qBSR9-kc increased resistance. The major QTL qBSR11-kc explained 23.0%–25.9% of the total phenotypic variation. Two QTLs (qBSR9-kc and qBSR11-kc) were detected in both years, whereas the other two were detected only in 2016. Genetic markers flanking these four QTLs will be powerful tools for marker-assisted selection to improve BS resistance.

Key Words: Bipolaris oryzae, brown spot, Oryza sativa L., QTL analysis, resistance, rice.
Materials and Methods

Fungal strain

Bipolaris oryzae strain Iga-2 (deposited as MAFF 245177 at the Genebank Project of National Agriculture and Food Research Organization (NARO), Tsukuba, Japan) was used. Inoculation, culture of the mycelia, and induction of conidiophore formation under irradiation with black-light lamps were based on the methods of Kihara and Kumagai (1994).

Plant materials

The BS-resistant donor, Oryza sativa L. ssp. indica, cv. CH45 (acc. no. JP12893), was provided by the Gene Bank Project, NARO. BILs were developed by first crossing CH45 with the susceptible parent O. sativa L. ssp. japonica, cv. Koshihikari. An F₁ plant was backcrossed to Koshihikari and 60 BC₁F₁ plants were obtained. Successive backcrossing with Koshihikari was performed to produce 229 BC₂F₁ seeds. Generations were advanced by means of single-seed descent. A population of 190 BILs at the BC₃F₅ generation was obtained in 2015.

Field evaluation of brown spot resistance

In 2015 and 2016, BS resistance in the BIL population was evaluated for QTL mapping in a research field at the Mie Prefecture Agricultural Research Institute (MPARI, Iga, Mie, Japan) with two replications, according to Matsumoto et al. (2016). Spreader plants inoculated with the fungus were planted around experimental plots as described by Matsumoto et al. (2016). Disease scores using a scale from 0 (no incidence) to 9 (severe) were recorded 108 and 105 days after transplanting in 2015 and 2016, respectively.

Linkage and QTL analysis

Total DNA was extracted from leaves by using the CTAB method (Murray and Thompson 1980). To construct a linkage map, we used 126 polymorphic rice SSR markers. SSR analysis was carried out according to McCouch et al. (2002). Linkage groups and marker order 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, Map Draw (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, standard model 6, five control markers, a 10-cM window size, and the forward and backward regression model. We used the genome-wide threshold value α = 0.05 to detect putative QTLs based on the results of 1000 permutations.

Results

Phenotypic analysis of parental lines and their progeny

Disease severity of BS at MPARI was greater in 2016 than in 2015, but distinct differences in BS field resistance were observed between the parental cultivars, CH45 and Koshihikari. The mean disease score of CH45 was 2.0 in 2015 and 2.6 in 2016 and that of Koshihikari was 4.1 in 2015 and 5.9 in 2016 (Figs. 1, 2). The disease scores of the BIL population in 2015 and 2016 were normally distributed, and some lines transgressively segregated in both directions (Fig. 2), indicating quantitative inheritance of field resistance. Significant negative correlations (P < 0.01) between disease scores and days to heading (DTH) were found in the BIL population (r = –0.289 in 2015 and –0.373 in 2016) (data not shown).

QTLs for BS resistance

We used 126 SSR polymorphic markers for map construction. The ratio of genotypes in the population was as follows: 83.3% homozygous for Koshihikari, 10.3% homozygous for CH45, 6.0% heterozygous, and 0.4% missing data. Heterozygous regions remained in the BILs, but segregation without heterozygous regions fitted a ratio of 7/8 (homozygous for Koshihikari): 1/8 (homozygous for CH45), as expected in a BC₂ population. We constructed a linkage map for all chromosomes, which covered a total genetic distance of 427.2 cM (Fig. 3). The genome coverage of the map was 94% (350.5 Mb/373.2 Mb of Nipponbare pseudomolecule, IPGSP-1.0), as estimated from the physical positions of markers at the distal end of each chromosome (Fig. 3).
Thus, despite the correlation between the two traits, no DTH loci were identified on the same chromosomes as the disease resistance loci.

Discussion

No major genes conferring immunity to BS have been identified, but a few cultivars, such as CH45, have sufficiently high quantitative resistance that may be agriculturally useful (Eruotor 1985, Sato et al. 2008). In the present study, we conducted QTL analysis for BS quantitative resistance with a BIL population derived from a cross between CH45 and Koshihikari.

A negative correlation between disease score and DTH was observed in the population, but the genes controlling disease score and DTH are represented as black ovals and a striped oval, respectively.

Table 1. Putative QTLs for BS resistance and DTH

| Trait                  | Years | QTL     | Chromosome | Marker interval 1) | LOD score | Variance explained of total (%) | Additive effect 2) |
|------------------------|-------|---------|------------|-------------------|-----------|---------------------------------|-------------------|
| Brown spot resistance  | 2015  | qBSR9-kc| 9          | RM3919-RM6797     | 3.7       | 6.5                             | 0.2               |
|                        |       | qBSR11-kc| 11         | RM6534-RM4112     | 12.6      | 25.9                            | -0.5              |
|                        | 2016  | qBSR2-kc| 2          | RM5578-RM5672     | 5.8       | 17.1                            | -0.6              |
|                        |       | qBSR7-kc| 7          | RM1353-RM5672     | 3.0       | 7.8                             | -0.4              |
|                        |       | qBSR9-kc| 9          | RM3919-RM6797     | 3.3       | 6.3                             | 0.3               |
|                        |       | qBSR11-kc| 11         | RM6534-RM4112     | 10.1      | 23.0                            | -0.8              |
| Days to heading        | 2015, 2016 | qDTH4-kc| 4          | RM16739-RM5586    | 22.3      | 32.7                            | 34.6              |

1) The nearest markers are underlined.
2) Negative values mean that the ‘CH45’ allele decreased the disease score.

Four QTLs (qBSR2-kc, qBSR7-kc, qBSR9-kc, and qBSR11-kc) for BS resistance were identified on chromosomes 2, 7, 9, and 11 (Fig. 3, Table 1); qBSR11-kc had the highest logarithm of odds (LOD) score (12.6 in 2015 and 10.1 in 2016) and was considered as a major QTL. The alleles from CH45 explained 17.1% (qBSR2-kc), 7.8% (qBSR7-kc), and 23.0%–25.9% (qBSR11-kc) of the total phenotypic variation, whereas qBSR9-kc from Koshihikari accounted for 6.3%–6.5%. Two QTLs (qBSR9-kc and qBSR11-kc) were detected in both years, whereas the other two were detected only in 2016.

As significant negative correlations between disease scores and DTH were found in both years, we also mapped the QTL for DTH. A single QTL associated with DTH (qDTH-kc4) was mapped on chromosome 4 (Fig. 3, Table 1).
disease scores and DTH appear to map on different chromosomes (Fig. 3). Therefore, BS resistance is not a pleiotropic effect of delayed heading.

We identified a total of four QTLs for BS resistance on chromosomes 2, 9, and 11; each QTL explained 6.3% to 25.9% of the phenotypic variation (Fig. 3, Table 1). Except for qBSR9-kc, the resistant parent CH45 contributed the resistance alleles of these QTLs. Two QTLs (qBSR9-kc and qBSR11-kc) were stable in both years, whereas the other two (qBSR2-kc and qBSR7-kc) were detected only in 2016. Monthly average temperatures at MPARI from June to September 2016 were 1.0–2.3°C higher than in 2015, and heavy rainfalls were frequent in September 2016 (Japan Meteorological Agency 2016), which may have increased the disease severity in 2016 in comparison with 2015 and thus facilitated the detection of additional QTLs.

The BS-resistance QTLs on chromosomes 2, 9, and 11 were already reported (Katara et al. 2010, Sato et al. 2008, 2015). The marker intervals of these QTLs were similar to those of the QTLs detected in this study. To the best of our knowledge, qBSR7-kc on chromosome 7 is a novel QTL for BS resistance.

Our research group previously reported the QTLs qBS2, qBS9, and qBS11 (the latter is the same as the major QTL qBSR11) for BS resistance in RILs derived from a cross between Tadukan ( indica) and Hinohikari ( japonica); the resistance alleles at qBS9 and qBS11 were provided by the indica parent and at qBS2 by the japonica parent (Sato et al. 2008, 2015). In the present study, we found that qBSR11-kc from the indica parent CH45 was a major BS resistance QTL; however, unlike in the previous study, the resistance allele at qBSR2-kc was from the indica parent and that at qBSR9-kc was from the japonica parent. The existence of different resistance alleles in the same QTL region may be important for the introduction of resistance QTLs from indica germplasm to materials with the japonica background through gene pyramiding in breeding programs. Now we are developing a program to breed practical japonica cultivars with strong BS resistance.

Acknowledgments

We thank Dr. T. Ando and Dr. S. Fukuoka (NARO, Japan) for their support during the SSR analysis. This work was supported by grants from the Ministry of Agriculture, Forestry and Fisheries of Japan (Project for “Development of mitigation and adaptation techniques to global warming in the sectors of agriculture, forestry, and fisheries”, Rice #1201 and #1401).

Literature Cited

Eruotor, P.G. (1985) Varietal reaction of rice to isolates of Cochliobolus miyabeanus. Indian Phytopath. 39: 62–64.

Japan Meteorological Agency (2016) Tables of monthly climate statistics. http://www.data.jma.go.jp/obd/stats/data/en/smp/index.html

Yamaguchi, Y., K. Nakano and R. Saito (2007) An outbreak of rice brown spot at Niigata prefecture Kaetsu district in 2005 and 2006. In: Japan Plant Protection Association (eds.) Cata- logue of agricultural chemicals, Tokyo, pp. 596–599.

Katara, J.L., H. Sonah, R.K. Deshmukh, R. Chaurasia and A.S. Kotasthane (2010) Molecular analysis of QTLs associated with resistance to brown spot in rice (Oryza sativa L.). Indian J. Genet. 70: 17–21.

Kihara, J. and T. Kumagai (1994) Ecotypes of the fungus Bipolaris oryzae with various responses of the mycochrome system. Physiol. Plant. 92: 689–695.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln and L. Newburg (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174–181.

Liu, H. and J.L. Meng (2003) MapDraw: a Microsoft Excel macro for drawing genetic linkage maps based on given genetic linkage data. Yi Chuan 25: 317–321.

Matsumoto, K., H. Sato, C. Ota, S. Seta, T. Yamakawa, H. Suzuki and Y. Nakayama (2016) A new method for evaluating field resistance to brown spot in rice. Breed. Res. 18: 103–111.

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.

Ministry of Agriculture, Forestry and Fisheries (2015) Climate change adaptation plan of Ministry of Agriculture, Forestry and Fisheries. http://www.maff.go.jp/j/kanbo/kangyo/seisaku/pdf/pdf/tekiou_eng.pdf

Misra, A.K. (1985) Effect of intercepting populations of resistant cultivars on reducing brown spot disease build up in a susceptible rice cultivar. Indian Phytopath. 38: 66–69.

Mizobuchi, R., S. Fukuoka, S. Tsushima, M. Yano and H. Sato (2016) QTLs for resistance to major rice diseases exacerbated by global warming: brown spot, bacterial seedling rot, and bacterial grain rot. Rice 9: 23.

Murray, M.G. and W.F. Thompson (1980) Rapid isolation of high-molecular-weight plant DNA. Nucleic Acids Res. 8: 4321–4325.

Niigata Agricultural Research Center (2010) Silicon amendments stabilized the effect of fungicide to Brown spot disease in rice. Niigata Prefecture agriculture forest and fisheries research results informa- tion. http://www.ari.pref.niigata.jp/nourinsui/seika10/

Ohata, K. and C. Kubo (1974) Studies on the mechanism of disease resistance of rice varieties to Cochliobolus miyabeanus. Bull. Shikoku Agric. Exp. Stn. 28: 17–57.

Padmanabhan, S.Y. (1973) The great Bengal famine. Annu. Rev. Phytopathol. 11: 11–24.

Sato, H., I. Ando, H. Hirabayashi, Y. Takeuchi, S. Arase, J. Kihara, H. Kato, T. Imbe and H. Nemoto (2008) QTL analysis of brown spot resistance in Rice (Oryza sativa L.). Breed. Sci. 58: 93–96.

Sato, H., K. Matsumoto, C. Ota, T. Yamakawa, J. Kihara and R. Mizobuchi (2015) Confirming a major QTL and finding additional loci responsible for field resistance to brown spot (Bipolaris oryzae) in rice. Breed. Sci. 65: 170–175.

Wang, S., C.J. Basten and Z.-B. Zeng (2006) Windows QTL Cartogra- pher 2.5. Department of Statistics, North Carolina State University, Raleigh, NC, http://statgen.ncsu.edu/qtlcart/WQTLCart.htm. Cited 23 Oct. 2006.

Yamaguchi, Y., K. Nakano and R. Saito (2007) An outbreak of rice brown spot at Niigata prefecture Kaetsu district in 2005 and 2006. The Association for Plant Protection of Hokuriku 59: 4.