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Research Paper

Breeding and characterization of the world's first practical rice variety with resistance to brown spot (*Bipolaris oryzae*) bred using marker-assisted selection

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Brown spot (BS) caused by *Bipolaris oryzae* is a serious disease of rice and decreases grain yield. Breeding for BS resistance is an economical solution but has not hitherto been achieved. To develop a practical BS-resistant variety, we introduced a chromosomal segment including a quantitative trait locus (QTL) for BS resistance, *qBSfR11*, derived from the BS-resistant local resource 'Tadukan', into the genetic background of the high-yielding but susceptible 'Mienoyume'. Resistance is controlled by a single recessive gene in a 1.3-Mbp region. We named this gene *bsr1* (*brown spot resistance 1*). The near-isogenic line *bsr1*-NIL had a greater yield with larger grain width than Mienoyume but similar other agronomic traits in fields where BS was mild; it had a significantly lower BS disease score and a 28.8% higher yield in fields where BS was more severe, and it showed resistance to multiple isolates of BS fungus. It showed stable resistance to BS and had excellent agricultural traits in the presence of BS. We developed the *bsr1*-NIL with resistance to BS and applied it for variety registration to Ministry of Agriculture, Forestry and Fisheries in Japan as 'Mienoyume BSL'. This is the first report for the BS resistant rice variety bred using marker-assisted selection.

Key Words: *Oryza sativa* L., disease resistance, brown spot, *qBSfR11*, *bsr1*, near-isogenic line, breeding variety.

Introduction

Brown spot (BS) is a fungal disease that is caused by *Bipolaris oryzae* and infects various parts of rice plants. The incidence of grain yield losses by BS and the use of countermeasures to BS (e.g., silicon fertilization) in the USA and India have been reported (Barnwal *et al.* 2013, Datnoff *et al.* 1991). The rate of yield reduction is up to 20% (Chakrabarti 2001, Kamal and Mia 2009, Ou 1985). It is highly possible that BS will become a more serious disease under global warming because its optimal temperature range for growth of the pathogen is relatively high (Mizobuchi *et al.* 2016).

In Japan in 2019, BS infected 159,482 ha, the third-largest area after sheath blight (581,367 ha) and rice blast (451,197 ha) (JPPA 2021). During 1975–2019, the area peaked in 1984 (384,836 ha) and has since decreased, but it is gradually increasing again (JPPA 2020). In Niigata prefecture, a decrease in the application of fungicides because of expansion of the use of rice-blast-resistant lines (Ishizaki *et al.* 2005) is presumed to be a reason for the spread of BS (Yamaguchi *et al.* 2007). As many rice-blast-resistant varieties have now been developed, we need to pay more attention to BS.

Some local genetic resources such as 'Tadukan' (Ohata and Kubo 1974, Yoshii and Matsumoto 1951), 'CH45' (Misra 1985), 'Dawn' (Eruotor 1986), and 'Tetep' (Eruotor 1986, Ohata and Kubo 1974, Yoshii and Matsumoto 1951) are resistant to BS, and some quantitative trait loci (QTLs) for resistance have been detected (Mizobuchi *et al.* 2016). We detected a major BS resistance QTL, *qBSfR11*, on chromosome (Chr.) 11 by field resistance tests with recombinant inbred lines derived from crosses between the resistant 'Tadukan' and the susceptible 'Hinohikari' (Sato *et al.* 2015). The Tadukan allele at the QTL also conferred BS resistance in the 'Koshihikari' background (Sato *et al.* 2015). Two other QTLs were detected near *qBSfR11*: *BSq11.2v*, derived from IR62266 (Katara *et al.* 2010), and

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qBSR11-kc, from 'CH45' (Matsumoto *et al.* 2017b). However, very few QTLs or genes for resistance to BS have been reported, and there are no reports of the development of BS-resistant varieties by using resistance QTLs.

The aim of this study was to develop a practical variety with resistance to BS. In Mie prefecture, the high-yielding *japonica* variety 'Mienoyume' is grown on about 800 ha of paddy fields. It has resistance to rice blast owing to the presence of the resistance gene *Pita-2*, but not to BS (Yamakawa *et al.* 2002). In our previous study, Mienoyume was one of the varieties most susceptible to BS of about 140 accessions including NIAS core collections of Japanese rice landraces and world rice (Matsumoto *et al.* 2017a). Because Mienoyume has high yield and good grain appearance, we developed near-isogenic lines (NILs) of it with BS resistance. We bred 'Mienoyume BSL', which is the world's first practical variety with resistance to BS bred using marker-assisted selection (MAS). We discuss the stability of resistance to multiple BS strains.

Materials and Methods

Breeding of NILs

Fig. 1 shows the breeding scheme used for the development of the NILs. The donor parent (R307-48-9) was a NIL developed by Sato *et al.* (2015), in which the major resistance QTL (*qBSfR11*) on Chr. 11, derived from *indica* 'Tadukan' (resistant), had been introduced into the genetic background of 'Koshihikari' (susceptible). *qBSfR11* was transferred into the Mienoyume background by sequential backcrossing method. During backcrossing or selfing from 2014 to 2015, promising individuals or lines were selected by MAS with simple sequence repeat (SSR) markers (McCouch *et al.* 2002) based on the target region on Chr. 11. In 2016, six SSR markers at the *qBSfR11* locus were used to verify the size of the substituted segments. In 2017, we evaluated the BS resistance of 52 NILs (19 BC_5F_3, 33 BC_4F_4), divided into 12 groups based on their generations and genotypes, by field evaluation testing described later (**Fig. 2A**). The whole genome of six resistant NILs (one in group-9, two in group-10, three in group-11) was surveyed by using 243 single-nucleotide polymorphism (SNP) markers distributed evenly across the 12 chromosomes (Nagasaki *et al.* 2010, **Supplemental Table 1**). A BS-resistant NIL, named *bsr1*-NIL, was selected as a promising line. In 2018, three more SSR markers (RM27159, RM27163, and RM27244), located downstream of the six SSR markers used in 2016, were used to determine the genotype of *bsr1*-NIL in the *qBSfR11* region and to delimit the chromosomal location of the BS-resistance gene in a group-11 NIL with the shortest Tadukan segment among the BS-resistant lines.

Field trials

Field trials were conducted in the paddy fields at Mie Prefecture Agricultural Research Institute (Mie, Japan) in Matsusaka (34°63′N, 136°48′E) and Iga (34°70′N, 136°13′E).

BS resistance was evaluated in Iga on a scale of 0 (no incidence) to 9 (severe) according to the procedure of Matsumoto *et al.* (2016) by using *B. oryzae* strain Iga-2 (Acc. No. 245177, MAFF Genebank) with two or three replications. In 2016, the inheritance mode of BS resistance was evaluated by using 153 individuals in the BC_4F_2 generation derived from one BC_4F_1 individual that had been confirmed to be heterozygous at the *qBSfR11* locus by using SSR marker RM27073 (McCouch *et al.* 2002). We investigated their RM27073 genotype and BS resistance by field evaluation testing.

Other tests of agronomic traits of *bsr1*-NIL were conducted in Iga and Matsusaka, with two replications in 2018 and three replications in 2019 and 2020. *bsr1*-NIL and Mienoyume were transplanted on 14 or 15 May in Matsusaka and on 10 or 11 May in Iga at four seedlings per hill in 120 hills and six rows per replication, with a spacing of 30 cm × 15 cm in 2018 and 2019 and 30 cm × 18 cm in 2020. Nitrogen fertilizer was applied at 4.8 g N m^{-2} at transplanting and 4.0 g m^{-2} at heading in Matsusaka, and at 5.6 and 3.4 g m^{-2}, respectively, in Iga. Major agronomic traits (days to heading, culm length, panicle length, brown rice yield, panicle number, 1000-grain weight, brown rice protein content, grain appearance, grain shape) were measured each year. Days to heading was calculated as days from transplanting to heading. From the results of these trials, *bsr1*-NIL was confirmed as a candidate for a practical BS-resistant variety for its high yield.

In 2020, to evaluate the effect of *qBSfR11* on agronomic traits, we grew *bsr1*-NIL and Mienoyume in a part of the test field where BS was more severe after heading. Seedlings were transplanted on 28 May at four seedlings per hill in 80 hills and four rows per replication (three replications), with a spacing of 30 cm × 15 cm. Slow-release N fertilizer was applied at 7.5 g N m^{-2} at transplanting. Spreader plants (Mienoyume inoculated with Iga-2 strain) were planted around the plots but not within them. Plant height, stem number, and leaf greenness (SPAD value) were measured as indicators of crop growth at the panicle formation stage, when BS had not yet occurred. SPAD values were measured with a SPAD-502Plus chlorophyll meter (Konika Minolta, Inc., Tokyo, Japan). Yield, yield components (panicle number, spikelet number per panicle, percentage of filled spikelets, and 1000-grain weight), brown rice protein content, and grain appearance were measured at maturity.

Inoculation test using multiple BS strains

In 2020, *bsr1*-NIL, Mienoyume (susceptible), and Tadukan (resistant) were grown in 5.5 cm × 15.0 cm × 9.5 cm containers filled with sterilized soil (Clean No. 2, Ibiko Corporation, Gifu, Japan) inside a greenhouse of Mie Prefecture Agricultural Research Institute. Five seeds of each were sown in a row, at four rows per container. The isolates of BS fungus used were *B. oryzae* T. AOKI AR0126 (isolated

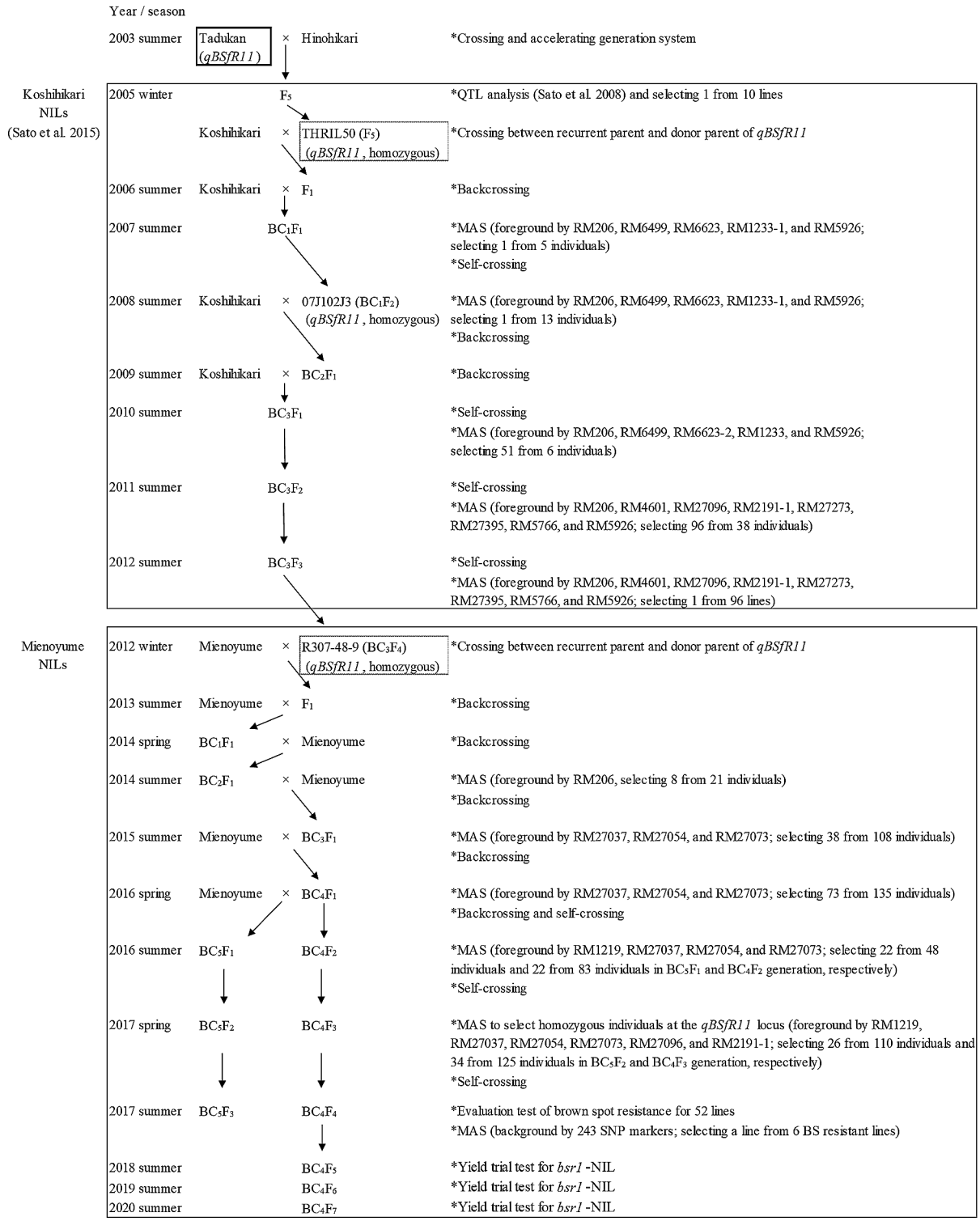

Fig. 1. Breeding scheme for development of Mienoyume NILs.

in Okinawa prefecture; MAFF Genebank Acc. No. 235499) and F-1 (isolated in Ehime prefecture; Acc. No. 305067). Inoculation of fungus and evaluation of disease symptoms followed the methods of Sato *et al.* (2008).

DNA isolation and marker analyses

Total DNA was extracted from the leaves by using the CTAB method (Murray and Thompson 1980). PCR and electrophoresis for SSR analyses followed the method of Sato *et al.* (2015), but with Taq enzyme from GoTaq Green Master Mix (Promega, Madison, WI, USA), 55°C annealing

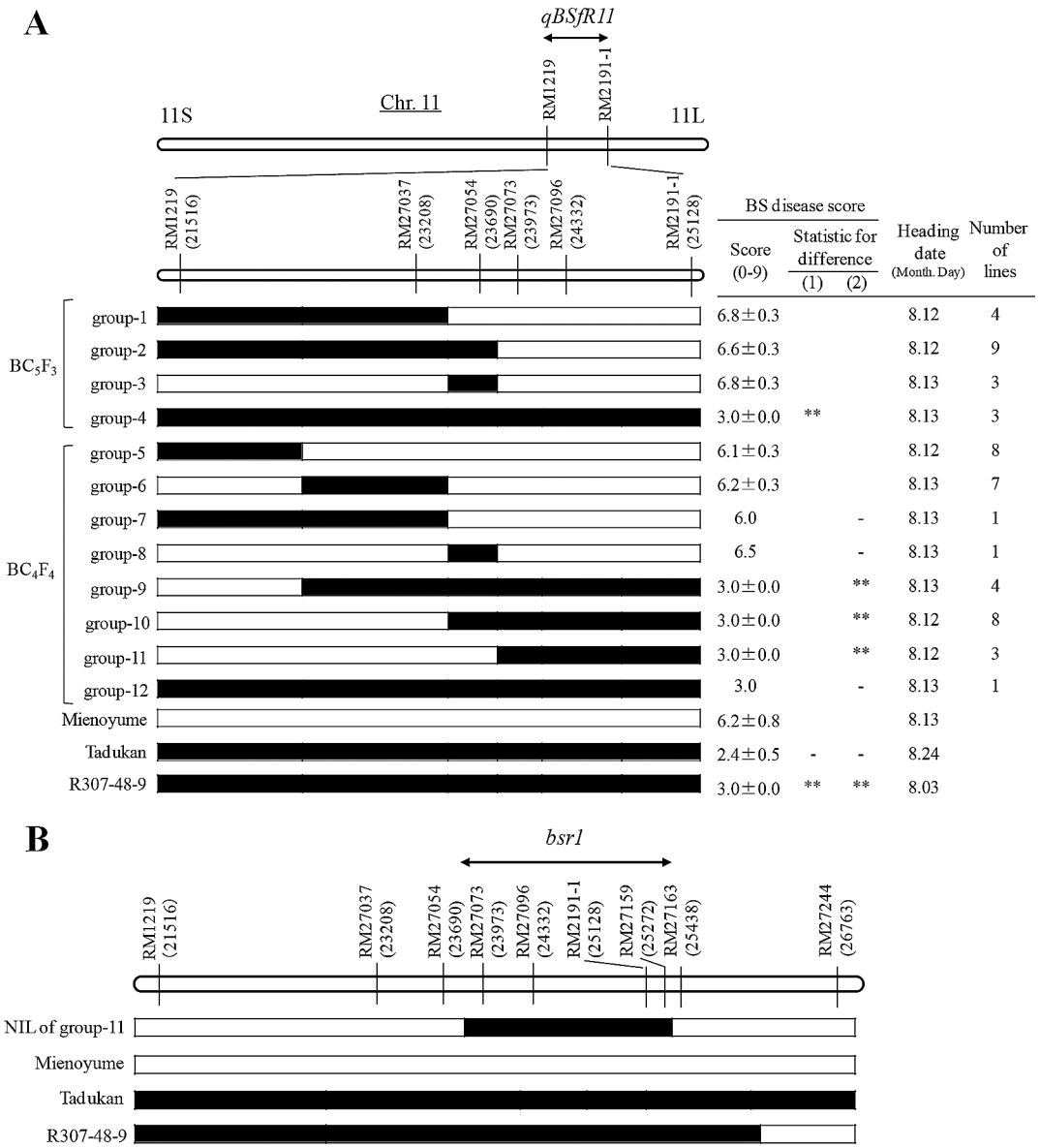

Fig. 2. Graphical genotypes in the *qBSfR11* region on Chr. 11 by SSR analyses in (A) 2016 and (B) 2018. □ Homozygous for Mienoyume; ■ homozygous for Tadukan. Numbers in parentheses beside SSR markers indicate their physical map positions (kbp) on Chr. 11 in IRGSP v. 1.0. A: 52 NILs in BC_5F_3 or BC_4F_4 generation with their BS disease scores (means ± SD) and heading dates in 2017. **Significant difference from Mienoyume at 1% in (1) BC_5F_3 and (2) BC_4F_4 generations (except in groups with one line) by Dunnett's test. There was no significant difference in heading dates between NILs (both generations) and Mienoyume at 5% by Dunnett's test. B: NIL of group-11.

temperature, and 3.0% gel concentration. All experimental procedures for the SNP analysis followed the method of Sato *et al.* (2015).

Results

Graphical representation of NIL genotypes

Fig. 2A shows the graphical genotypes at the *qBSfR11* region (between RM1219 and RM2191-1) and the phenotypes (BS disease scores and heading dates) of 52 NILs (BC_5F_3 19 lines, BC_4F_4 33 lines) that had been confirmed to be homozygous for either the Tadukan allele or the Mienoyume allele between RM1219 and RM2191-1 by SSR analysis in 2016. The genotype of the donor parent (R307-48-9) was the same as that of Tadukan (*qBSfR11* donor), and its BS disease score was significantly lower than that of Mienoyume. In both the BC_5F_3 generation and the BC_4F_4 generation, the groups with Mienoyume segments from RM27073 to RM2191-1 (groups-1, 2, 3, 5, 6, 7, and 8) had the same disease scores as Mienoyume. In contrast, the groups with Tadukan segments there (groups-4, 9, 10, 11, and 12) had lower disease scores than Mienoyume. The heading dates of all 52 NILs were the same as that of Mienoyume. SSR analysis downstream of RM2191-1 in

2018 showed that a NIL of group-11 had a 1.3-Mbp Tadukan segment from RM27073 to RM27159 (**Fig. 2B**).

Inheritance mode of BS resistance

Fig. 3 shows the frequency distribution of BS disease scores in 153 BC_4F_2 individuals, based on the genotypes of SSR marker RM27073 at the *qBSfR11* locus. Disease scores of 0 to 4 were considered to indicate resistance and those of 4.5 to 9 to indicate susceptibility. The BC_4F_2 individuals segregated in a 1:3 ratio of resistant: susceptible (**Table 1**), confirming that the resistance to BS is controlled by a single recessive gene at the *qBSfR11* locus. We named this gene *bsr1* (*brown spot resistance 1*).

Genetic backgrounds and agronomic traits of bsr1-NIL

We selected a resistant NIL (*bsr1*-NIL) in the BC_4F_4 generation in 2017. *bsr1*-NIL had the group-9 genotype (**Fig. 2A**). It had R307-48-9 segments on Chr. 11 (3.5 Mbp from aa 11004652 to aa 11007953; **Fig. 4C**). On all other chromosomes except for Chr. 11, it was homozygous for Mienoyume segments. The other five lines tested for the whole-genome survey had R307-48-9 segments on all other chromosomes except for Chr. 11.

In both yield-trial paddy fields where BS was less severe than in the BS resistance test field, there was no significant difference in BS disease score between *bsr1*-NIL and Mienoyume (**Table 2**). However, some traits were significantly different. In Matsusaka, brown rice yield and grain width of *bsr1*-NIL were 34 g m^{-2} higher and 0.06 mm larger, respectively, than those of Mienoyume. In Iga, grain width of *bsr1*-NIL was 0.06 mm larger than that of Mienoyume. In a part of the BS resistance test field where BS was more severe, the BS disease score of *bsr1*-NIL was 3.0 lower than that of Mienoyume (**Table 3**, **Fig. 4A**, **4B**). There were no significant differences in growth characteristics at the panicle formation stage between *bsr1*-NIL and Mienoyume. On the other hand, brown rice yield and percentage of filled spikelets of *bsr1*-NIL were respectively 106 g m^{-2} and 12.3% higher than those of Mienoyume. The protein content of brown rice of *bsr1*-NIL was 1.3% lower than that of Mienoyume. In addition, comparing agronomic traits of *bsr1*-NIL and Mienoyume in BS resistance test field (severe conditions) and yield-trial field (mild conditions), in 'severe conditions', both of them had lower brown rice yield and percentage of filled spikelets and higher protein content of brown rice than in 'mild conditions', and those degree of decrease or increase was smaller in *bsr1*-NIL than in Mienoyume. 1000-grain weight and grain width were also lower in 'severe conditions', but those degree of decrease was same in *bsr1*-NIL and Mienoyume. This showed that *bsr1*-NIL had larger 1000-grain weight with larger grain width than Mienoyume regardless of the BS severity. The same result was also found in **Table 2**. Panicle number and spikelet number per panicle were different between two fields, but this result is presumed not to be due to BS because they are the yield components determined before the heading stage when BS began to be severe in this study. These results suggest that BS reduced the ripening of rice and decreased the brown rice yield, and *qBSfR11* introduced into *bsr1*-NIL had the effect of reducing the decrease in brown rice yield by suppressing the decrease in percentage of filled spikelets by BS.

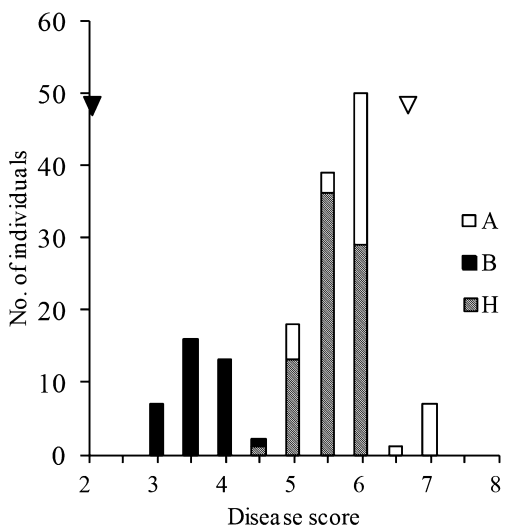

Fig. 3. Frequency distribution of BS disease scores in 153 BC_4F_2 individuals derived from one BC_4F_1 individual and based on the genotypes of a SSR marker RM27073. Bars: □ homozygous for Mienoyume allele (A), ■ homozygous for Tadukan allele (B), and ▨ heterozygous (H). Triangles denote disease scores of ▽ Mienoyume (6.7: susceptible) and ▼ Tadukan (2.0: resistant).

Table 1. The segregation of BC_4F_2 individuals as resistant or susceptible to BS

Generation	Number of individuals		χ^2-value (1:3)	*p*-value
	Resistant type	Susceptible type		
BC_4F_2	36	117	0.18	0.67

Resistance of bsr1-NIL to other isolates of BS fungus

At the seedling stage, the disease score of *bsr1*-NIL was significantly lower than that of Mienoyume following artificial inoculation of *B. oryzae* T. AOKI AR0126 and F-1 (**Table 4**). Thus, *bsr1*-NIL showed resistance to multiple isolates of BS fungus.

Discussion

Characteristics of BS-resistance QTL, qBSfR11

Mienoyume is highly susceptible to BS and is more susceptible than Koshihikari (Matsumoto *et al.* 2017a). Here, we developed NILs with resistance to BS by using *qBSfR11*, derived from Tadukan, which had been identified as a major QTL responsible for resistance to BS (Sato *et al.* 2015). *qBSfR11* had been previously confirmed to confer BS resistance in the Koshihikari genetic background (Sato

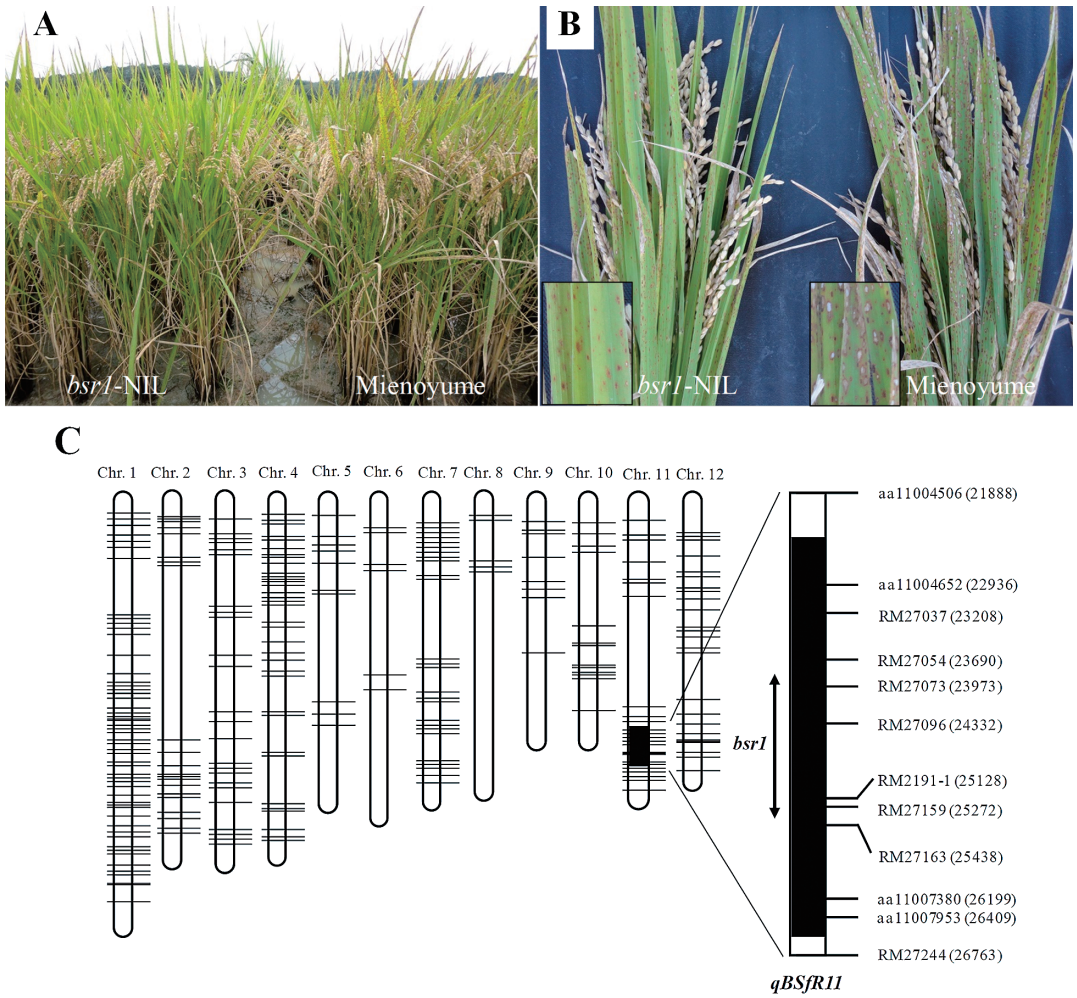

Fig. 4. Phenotype and genotype of *bsr1*-NIL. A: Test plot in the BS test field. B: BS lesions appeared on each plant. C: Graphical genotype. □ Homozygous for Mienoyume; ■ homozygous for R307-48-9. Numbers in parentheses beside markers indicate their physical map positions (kbp) on Chr. 11 in IRGSP v. 1.0. Detailed information of SNP markers used for this mapping is shown in **Supplemental Table 1**.

et al. 2015). Here, it conferred resistance in the Mienoyume genetic background also (**Table 3**, **Fig. 4**). This result strongly indicates that *qBSfR11* is effective at conferring resistance to BS.

The genotype of a resistant NIL in group-11 showed that *qBSfR11* was located around the 1.3-Mbp interval RM27073 to RM27159 (**Fig. 2B**). The candidate genomic region was narrowed from the donor parent R307-48-9. Annotation of the 'Nipponbare' sequence in RAP-DB shows 107 genes predicted within this interval (Sakai *et al.* 2013). It is hard to predict which of the genes might be related to BS resistance because there have been no reports of genes associated with BS resistance and because the morphological and physiological functions of *qBSfR11* are not yet known. Thus, further delimitation of the candidate genomic region of *qBSfR11* will be necessary to identify the gene corresponding to *qBSfR11*.

The distribution of BS disease scores in 153 BC_4F_2 individuals suggested that resistance to BS is controlled by a single recessive gene (**Fig. 3**). We named this gene *bsr1* (*brown spot resistance 1*). Mwendo *et al.* (2017) reported that resistance to BS was controlled by one or two dominant genes, whereas Adair (1941) reported the involvement of several recessive genes. These present and previous studies show that there are different genes for BS resistance, with different modes of inheritance. Goel *et al.* (2006) suggested that pyramiding QTLs for BS resistance would be effective because the resistance in four lines of wild rice *Oryza nivara* showed quantitative inheritance. In future work, *qBSfR11* should be an effective QTL for pyramiding to enhance BS resistance.

Characteristics of bsr1-NIL

By marker-assisted selection of foreground and background and BS resistance, *bsr1*-NIL was selected as a candidate for a practical variety with resistance to BS.

Bipolaris oryzae is genetically diverse in Bangladesh (Kamal and Mia 2009), the Philippines (Burgos *et al.* 2013), India (Archana *et al.* 2014), and Iran (Ahmadpour *et al.* 2018). Inoculation of seedlings of 80 rice varieties with

Table 2. Agronomic traits of *bsr1*-NIL and Mienoyume in 3 years (2018–2020)

Test site	Line or variety	BS disease score (0–5)	Days to heading (days)	Heading date	Ripening date	Culm length (cm)	Panicle length (cm)	Brown rice yield (g m^{-2})	Panicle number (m^{-2})	1000-grain weight (g)	Protein content of brown rice (%)	Grain appearance (%)	Grain shape		
													Grain length (mm)	Grain width (mm)	Grain length to width ratio
Matsusaka	*bsr1*-NIL	0.0 ± 0.1	81.3 ± 2.5	8.04	9.06	72.1 ± 5.4	20.8 ± 0.5	639 ± 12	400.6 ± 47.2	23.0 ± 0.8	7.0 ± 0.5	76.1 ± 14.4	5.07 ± 0.04	2.75 ± 0.03	1.85 ± 0.01
	Mienoyume	0.8 ± 1.1	81.3 ± 2.5	8.04	9.06	73.4 ± 6.2	20.8 ± 0.5	605 ± 14	388.4 ± 39.3	22.5 ± 0.8	7.2 ± 0.6	77.8 ± 10.6	5.08 ± 0.18	2.69 ± 0.02	1.89 ± 0.07
	t-test			–	–			*						*	
Iga	*bsr1*-NIL	0.4 ± 0.5	86.3 ± 3.2	8.04	9.11	72.0 ± 7.1	20.5 ± 0.7	670 ± 30	398.5 ± 21.4	23.9 ± 0.3	6.6 ± 0.1	89.7 ± 0.9	5.04 ± 0.02	2.72 ± 0.03	1.85 ± 0.02
	Mienoyume	2.1 ± 1.4	86.3 ± 3.2	8.04	9.11	74.4 ± 6.9	20.6 ± 0.6	635 ± 25	399.3 ± 13.0	23.4 ± 0.1	6.9 ± 0.0	91.5 ± 0.8	5.06 ± 0.08	2.66 ± 0.02	1.90 ± 0.04
	t-test			–	–					$p = 0.06$	*	$p = 0.06$		*	

Values of each agronomic trait are shown as means ± SD over 3 years. BS disease score was ranked on a scale of 0 (no incidence) to 5 (severe) by visual survey at maturity, different from the method of Matsumoto *et al.* (2016). Yield and 1000-grain weight were calculated from filled grains screened through a 1.85-mm-mesh sieve, at a moisture content of 15%. Protein content of brown rice is expressed on a dry-weight basis of filled grains evaluated by near-infrared reflectance spectroscopy (6500HON, Nireco, Tokyo, Japan). Grain appearance is the ratio of perfect grains to filled grains, evaluated by a grain rice quality inspector (RN500, Kett, Tokyo, Japan). Length and width of 1000 filled grains were measured with a Satake Rice Analyzer (RGQI10B, Satake, Hiroshima, Japan). *Significant at 5%. $p < 0.10$ is indicated.

Table 3. Agronomic traits of *bsr1*-NIL and Mienoyume in fields with different degree of BS in 2020

Test site (Degree of BS)	Line or variety	BS disease score (0–9)	Heading date	Ripening date	At the panicle formation stage			Yield and yield components						Protein content of brown rice (%)	Grain appearance (%)	Grain shape	
								Brown rice yield									
					Plant height (cm)	Stem number (m^{-2})	SPAD value	Yield (g m^{-2})	Yield comparision (%)	Panicle number (m^{-2})	Spikelet number per panicle (/panicle)	Percentage of filled spikelets (%)	1000-grain weight (g)			Grain length (mm)	Grain width (mm)
BS resistance test field (Severe)	*bsr1*-NIL	4.0 ± 0.0	8.17	9.14	79.9 ± 2.1	565.9 ± 41.1	36.1 ± 2.5	474 ± 42	128.8	352.7 ± 2.6	87.3 ± 3.2	67.1 ± 6.4	22.7 ± 0.2	7.7 ± 0.1	91.1 ± 1.4	4.81 ± 0.11	2.67 ± 0.01
	Mienoyume	7.0 ± 0.0	8.17	9.14	81.8 ± 1.1	600.7 ± 60.0	36.8 ± 0.8	368 ± 18	100.0	383.6 ± 18.5	82.1 ± 10.2	54.8 ± 3.1	22.3 ± 0.0	9.0 ± 0.6	87.8 ± 1.7	5.06 ± 0.07	2.59 ± 0.01
	t-test	***						*				*	$p = 0.06$	*	$p = 0.06$	*	***
Yield-trial field (Mild)	*bsr1*-NIL	1.0 ± 0.0	8.12	9.09	–	–	–	703 ± 6	106.2	390.1 ± 4.9	83.6 ± 2.1	89.0 ± 1.9	24.0 ± 0.0	6.8 ± 0.1	90.8 ± 0.4	5.02 ± 0.02	2.72 ± 0.01
	Mienoyume	3.0 ± 0.0	8.12	9.09	–	–	–	662 ± 37	100.0	378.2 ± 15.9	84.4 ± 3.8	88.2 ± 1.6	23.3 ± 0.1	6.8 ± 0.2	92.2 ± 2.1	5.05 ± 0.02	2.65 ± 0.01
	t-test	***											***				**
Severe/Mild (%)																	
	bsr1-NIL	–	–	–	–	–	–	67	–	90	104	75	95	113	100	96	98
	Mienoyume	–	–	–	–	–	–	56	–	101	97	62	96	132	95	100	98

Values of each agronomic trait are shown as means ± SD of three replications in 2020. Both fields were set up in Iga. Yield and 1000-grain weight were calculated from filled grains screened through a 1.85-mm-mesh sieve, at a moisture content of 15%. Brown rice yields were compared as that of *bsr1*-NIL divided by that of Mienoyume. Percentage of ripened spikelets was calculated as number of filled spikelets divided by total number of spikelets. Protein content of brown rice is expressed on a dry-weight basis of filled grains evaluated by near-infrared reflectance spectroscopy (6500HON, Nireco, Tokyo, Japan). Grain appearance is the ratio of perfect grains to filled grains, evaluated by a rice grain quality inspector (RN500, Kett, Tokyo, Japan). Length and width of 1000 filled grains were measured with a Satake Rice Analyzer (RGQI10B, Satake, Hiroshima, Japan). Significant at *5%, **1%,***0.1%. $p < 0.10$ is indicated.

Table 4. Disease resistance reactions to two BS isolates under artificial inoculation in the greenhouse

Line and variety	Disease score	
	T. AOKI AR0126	F-1
bsr1-NIL	2.7 ± 0.6 b	2.0 ± 0.0 b
Mienoyume	4.3 ± 0.6 c	5.0 ± 0.0 c
Tadukan	1.0 ± 0.0 a	1.0 ± 0.0 a
ANOVA	***	***

Disease scores are shown as means ± SD. ***Significant at 0.1%. Values followed by the same letter within a column are not significantly different at 5% by Tukey–Kramer test.

107 *B. oryzae* isolates collected from various regions in Japan revealed significant differences in pathogenicity; in some cases, the reaction was reversed depending on the combination of variety and isolate (Ohata 1989). Although race grouping of *B. oryzae* has not been reported so far, the existence of races is clear. Therefore, we tested whether *bsr1*-NIL was resistant to different *B. oryzae* strains. It showed resistance to *B. oryzae* T. AOKI AR0126 and F-1 (**Table 4**), as well as to *B. oryzae* Iga-2. This result suggests that BS-resistant NILs with *qBSfR11* will have resistance in different regions, at least in Japan.

The most important agronomic trait of the recurrent parent Mienoyume is its high yield. In Mie prefecture, the brown rice yield is about 600 g m^{-2} higher than that of Japan's most famous variety, Koshihikari (Kobayashi *et al.* 2018). *bsr1*-NIL had a higher yield than Mienoyume (**Table 2**). In addition, the results suggest that there may be useful new genes related to grain width and derived from Tadukan near *bsr1*; the higher yield of *bsr1*-NIL is likely to be due to the larger grain size. However, *bsr1*-NIL had the same grain length and grain length–width ratio as Mienoyume. *bsr1*-NIL has Mienoyume segments in all chromosomal regions except for the *qBSfR11* region on Chr. 11. This shows that the homozygous Tadukan allele of *bsr1*-NIL in the *qBSfR11* region increased grain width (**Supplemental Fig. 1**). Near this region, *tgw11*, associated with grain weight and grain width, was previously reported (Oh *et al.* 2011), but the *tgw11* region did not overlap with the *qBSfR11* region in which *bsr1*-NIL had the homozygous Tadukan allele. On the other hand, the grain width of R307-48-9 (the donor parent of *bsr1*-NIL) was also slightly larger than that of its recurrent parent, Koshihikari. However, the difference is not significant (data not shown). It is quite likely that the expression of the grain width gene may change due to the difference in background genotypes of Koshihikari and Mienoyume. Further analysis of the *qBSfR11* region will be also necessary in terms of grain width. *bsr1*-NIL may have useful new genes related to grain width derived from Tadukan in the *qBSfR11* region. Thus, Tadukan may be the source of not only BS resistance, but also of the large grain width.

The impact of BS infection to agricultural traits and the effect of qBSfR11 in improving rice yield and quality

In the BS resistance test field, *bsr1*-NIL had 28.8% higher yield than Mienoyume (**Table 3**). We inferred that the reason was the higher percentage of filled spikelets and larger 1000-grain weight. There were no significant differences in growth characteristics between *bsr1*-NIL and Mienoyume at the panicle formation stage, when BS was mild. BS became more severe after heading, and so is likely to affect ripening. In addition, it was presumed that the higher percentage of filled spikelets was the effect of *qBSfR11* of suppressing BS and the larger 1000-grain weight was the effect of new genes related to grain width in the *qBSfR11* region. The protein content of brown rice of *bsr1*-NIL was significantly lower than that of Mienoyume. Vidhyasekaran and Ramadoss (1973) reported that severe infection reduced both yield (~20% to 40%) and quality (i.e., increased protein content), as here. Dallagnol *et al.* (2014) reported that BS reduced yield by reducing grain number per panicle, 1000-grain weight, and the percentage of filled grains. Aluko (1975) reported that severe infection reduced grain number per panicle and individual grain weight, resulting in a yield loss of 30% to 43%, compared with only 12% under moderate infection. The BS pathogen attacks the rice plant from seedling to milk stage (Sunder *et al.* 2014). The degree of yield loss and contributing factors are thought to vary depending on the degree and timing of BS infection. If BS is serious at an earlier stage than here, there is a high possibility that BS will affect not only ripening, but also yield components such as panicle number, and damage will be greater. Because BS resistance QTL, *qBS11*, which detected in the same region as *qBSfR11* (Sato *et al.* 2015), has resistance to BS at the seedling stage of rice (Sato *et al.* 2008), *bsr1*-NIL is expected to have resistance even if BS occurs at an earlier stage than here. On the other hand, *bsr1*-NIL had a lower yield and a higher protein content of brown rice (lower quality) in BS severe conditions than in mild conditions, although its yield decrease and protein content increase were smaller than those of Mienoyume (**Table 3**). As the resistance type of *bsr1*-NIL with *qBSfR11* is not true resistance but field resistance, pyramiding of QTLs is required for further enhancement of BS resistance.

First practical BS resistant variety bred using MAS

We submitted *bsr1*-NIL for variety registration with the Ministry of Agriculture, Forestry and Fisheries in Japan as 'Mienoyume BSL' (where BSL = Brown Spot resistance Line). This is the world's first practical BS resistant variety bred using MAS.

Author Contribution Statement

KM designed the research and wrote the manuscript; KM and YO mainly performed the experiments and analyzed data; TY, TO, YH performed phenotypic examinations of

NILs; SS selected individuals with MAS for foreground; RM and HS selected individuals and lines with MAS for foreground and background, and oversaw and improved manuscript.

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