

## Research Paper

# Evaluation of major rice cultivars for resistance to bacterial seedling rot caused by *Burkholderia glumae* and identification of Japanese standard cultivars for resistance assessments

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*Burkholderia glumae* causes bacterial seedling rot (BSR) and bacterial grain rot (BGR) in rice (*Oryza sativa*), both of which are important diseases in Japan. We previously evaluated major Japanese cultivars for BGR resistance and selected standard cultivars for resistance assessments. Here, we assessed the BSR occurrence rate in cultivars from the World Rice Collection (WRC) and other sources and found wide variation in resistance. Next, we evaluated major Japanese cultivars for BSR resistance and found that two Japanese landraces, ‘Kujuu’ and ‘Aikoku’, showed “strong” resistance; most others were categorized as “medium” or “medium to weak”. We previously developed a near-isogenic line (*RBGI-NIL*) by introducing the genomic region containing *RBGI*, a quantitative trait locus (QTL) for BSR resistance, from ‘Nona Bokra’ (*indica*) into ‘Koshihikari’ (*temperate japonica*). The resistance level of *RBGI-NIL* was “strong”, indicating the effectiveness of *RBGI* against BSR. The correlation between BSR and BGR resistance scores was low, indicating that it is necessary to introduce QTLs for resistance from different sources to develop cultivars resistant to both BSR and BGR. On the basis of the screening results, we selected standard cultivars for BSR resistance to cover a range of resistance levels.

**Key Words:** *Oryza sativa* L., disease resistance, bacterial seedling rot (BSR), standard cultivar, *Burkholderia glumae*, core collection, rice.

## Introduction

Climate change has widespread impacts on food production, and global warming is a serious problem. The global mean surface temperature may rise by up to 4.8°C by the end of this century relative to the period from 1986 to 2005 (IPCC 2013, Ishimaru *et al.* 2016). According to the Japan Meteorological Agency, the average temperature of east Japan in July of 2018 was the highest since statistical data were first collected in 1964 (JMA 2018). In rice (*Oryza sativa* L.), bacterial seedling rot (BSR) and bacterial grain rot (BGR), both caused by *Burkholderia glumae*, are two of the most serious diseases. These diseases are expected to increase in extent under global warming because the optimal temperature range for growth of the pathogen is relatively high (30–35°C) (Kurita *et al.* 1964, Tsushima *et al.*

1986). Since *B. glumae* was first discovered in 1955 in Japan (Goto and Ohata 1956, Goto *et al.* 1987, Kurita and Tabei 1967, Uematsu *et al.* 1976), it has been reported in other regions such as the USA (Nandakumar *et al.* 2009), East and South Asia (Ashfaq *et al.* 2017, Chien and Chang 1987, Cottyn *et al.* 1996a, 1996b, Jeong *et al.* 2003, Luo *et al.* 2007, Mondal *et al.* 2015, Trung *et al.* 1993), Latin America (Nandakumar *et al.* 2007, Zeigler and Alvarez 1989), and South Africa (Zhou 2014). Primary infection occurs when seeds contaminated with *B. glumae* are sown and BSR becomes apparent in the seedlings. After the infected plants are transplanted into fields, plants located near the diseased primary-infected plants are attacked by the pathogen at the heading stage, thus establishing secondary infection. The spikelet color changes from the normal green to reddish brown, and BGR becomes apparent. Eventually, the infection may cause unfilled or aborted grains (Ham *et al.* 2011), and the contaminated seeds cause BSR the following year. In 2017, the epidemic area of BGR in Japan covered 30 053 ha (JPPA 2018).

Although seed treatment with oxolinic acid, a quinoline derivative, has been a major means of bacterial disease

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control in Japan (Hikichi 1993a, 1993b, Hikichi *et al.* 1989), many strains of *B. glumae* tolerant to oxolinic acid have been found in several prefectures, for example in Toyama (Morikawa *et al.* 1997), Nagano (Yamashita *et al.* 1998), Iwate (Fukushi *et al.* 2000), Niigata (Hori *et al.* 2004), Fukushima (Minagawa and Yamada 2008), and Chiba (Ohtani and Takeuchi 2013). Therefore, the need for cultivars carrying genes for resistance to BSR and BGR is increasing to ensure sustainable rice production. However, cultivars with BSR and BGR resistance have not yet been developed in Japan. To date, only one QTL for resistance to BSR has been identified (Mizobuchi *et al.* 2013b, 2016, Zarbafi and Ham 2019). This QTL (*RBG1*; formerly named *qRBS1*) was found on chromosome 10 in chromosome segment substitution lines (CSSLs) derived from a cross between ‘Nona Bokra’ (resistant) and ‘Koshihikari’ (susceptible) (Mizobuchi *et al.* 2013b). On the other hand, several QTLs for resistance to BGR have been identified (Mizobuchi *et al.* 2013a, 2015, Pinson *et al.* 2010, Zarbafi and Ham 2019). A major QTL (*RBG2*) for BGR resistance was mapped on the long arm of chromosome 1 in backcross inbred lines (BILs) derived from a cross between ‘Kele’ (resistant) and ‘Hitomebore’ (susceptible) (Mizobuchi *et al.* 2013a, 2015).

Although the same organism (*Burkholderia glumae*) causes both BSR and BGR in rice, the number of reports of screening cultivars for resistance to BSR is smaller than that for BGR (Mizobuchi *et al.* 2016), and no resistant cultivars have been found in common by different groups, likely because of the different methods used. Reported BSR evaluation methods include injection of a bacterial suspension into the soil at germination (Goto 1982, 1983), inoculation of sterilized seeds (Hirashima and Wakimoto 1983, Maeda *et al.* 2016), and inoculation of seedlings by spray (Sayler *et al.* 2006) or needle (Wamishe *et al.* 2014). Because no highly resistant cultivars were identified in the studies that used soil inoculation or seedling inoculation by needle (Goto 1982, 1983, Wamishe *et al.* 2014), we tried inoculation of sterilized seeds (Hirashima and Wakimoto 1983, Maeda *et al.* 2016) and inoculation of seedlings by spraying (Sayler *et al.* 2006). We could not obtain consistent results with the spray inoculation method (data not shown), so here we inoculated sterilized seeds (Hirashima and Wakimoto 1983, Maeda *et al.* 2016).

Because we wanted to identify highly resistant cultivars for use in Japan, we conducted the evaluation in two steps. First, we assessed BSR occurrence in over 100 Japanese and world cultivars. Next, we evaluated major Japanese cultivars for BSR resistance using a less-severe inoculation method in order to detect small differences in resistance between cultivars. On the basis of the screening results, we selected standard cultivars for BSR resistance to represent a range of resistance levels. Because we had previously evaluated major Japanese cultivars for BGR resistance (Mizobuchi *et al.* 2018), we compared the results of the BSR and BGR resistance evaluations.

## Materials and Methods

### Plant materials

In 2008, 102 rice (*Oryza sativa* L.) cultivars (**Supplemental Table 1**) were grown in paddy fields at NARO in Tsukuba, Japan, and the seeds were harvested for evaluation of the BSR occurrence rate (see below). Seeds from the World Rice Collection of NARO (WRC) were obtained from the Gene Bank of the NIAS (now located at NARO). WRC cultivars were categorized into *indica*, *temperate japonica*, and *tropical japonica* (Ebana *et al.* 2010, Kojima *et al.* 2005). Because the Japanese Rice Collection of NARO (JRC) was under development at the beginning of the experiment, we could not obtain the full set of JRC lines, so representative landraces from JRC were selected for this experiment by Dr. K. Ebana (NARO).

The cultivars to be evaluated for BSR resistance (**Supplemental Table 2**) were grown in paddy fields at NARO in Tsukuba, Japan, in 2017, and the seeds were harvested for evaluation of BSR resistance (see below). Forty-three major Japanese rice cultivars previously analyzed for BGR resistance (Mizobuchi *et al.* 2018) were evaluated. Because Hokkaido is a cold region of Japan and the level of damage from BSR and BGR is low, cultivars from there were not included in this experiment. “Top 20” cultivars are Japanese non-glutinous cultivars; the 17 of “Top 20” cultivars included in this study (which excluded three cultivars from Hokkaido) together represented about 80% of the cultivated rice area in Japan in 2017 (<http://www.komenet.jp/pdf/H29sakutuke.pdf> [in Japanese]).

We previously mapped two QTLs, *RBG1* for BSR resistance and *RBG2* for BGR resistance. *RBG1* (*Resistance to Burkholderia glumae 1*), initially named *qRBS1*, was the first fine-mapped QTL related to BSR resistance (Mizobuchi *et al.* 2013b). We backcrossed the genomic region containing *RBG1* (about 380 kb) from ‘Nona Bokra’, a resistant traditional lowland *indica* cultivar that originated in India, into ‘Koshihikari’, a susceptible modern lowland *temperate japonica* cultivar from Japan, and named the near-isogenic line *RBG1-NIL*. *RBG2* (Mizobuchi *et al.* 2015) was detected in ‘Kele’, a BGR-resistant traditional lowland *indica* cultivar that originated in India. We introduced the genomic region containing *RBG2* (about 30 kb) from a resistant BIL into ‘Hitomebore’, a susceptible modern lowland *temperate japonica* cultivar from Japan, and named the near-isogenic line *RBG2-NIL* (Mizobuchi *et al.* 2018). In this experiment, we analyzed the BSR resistance of *RBG1-NIL*. Because we were still developing *RBG2-NIL* at the time, we could not analyze its BSR resistance together with other cultivars, but compared ‘Hitomebore’ and *RBG2-NIL* later.

### Assessment of BSR occurrence rate

BSR resistance was evaluated in two steps. The first measured BSR occurrence in over 100 Japanese and world

cultivars under high inoculation pressure, and the second measured BSR severity, primarily in Japanese cultivars, under lower inoculation pressure. The bacterial strain used in both steps was *B. glumae* MAFF 301682. In the first step, the bacterial inoculum suspension was adjusted to a concentration of  $10^8$  per mL with sterilized water. The inoculum preparation and inoculation were conducted as previously described (Mizobuchi *et al.* 2013b). Rice seeds were sterilized by soaking in chlorine bleach (available chlorine 2.5%) for 30 min and rinsed carefully with sterilized water. The sterilized seeds were placed in a freshly prepared bacterial suspension and held under vacuum ( $-0.02$  MPa) for 3 min. The inoculated seeds were dried overnight and then soaked in sterilized water for 2 days in a plant growth chamber at 27°C. The seeds were sown in sterilized soil (Bonsol No. 2, Sumitomo Kagaku Kougyo, Osaka, Japan), and incubated in a growth chamber at 27°C with 80% humidity under a 14-h photoperiod. Plant phenotypes were classified as “healthy” or “diseased” at 7–10 days after inoculation, and the BSR occurrence rate was calculated as the percentage of diseased plants, including ungerminated seeds, among the total (15) seeds used for inoculation. There were three replications per inoculation. Because many cultivars were examined, we grouped the cultivars according to cultivar type and other characteristics and inoculated them on seven different dates (**Supplemental Table 1**). The effectiveness of each inoculation test was confirmed by the inclusion of ‘Hitomebore’ (susceptible) as a recurrent control.

### Assessment of BSR resistance

Inoculum preparation and assessment of BSR resistance were conducted as above except that the inoculum concentration was lower in order to detect small differences in resistance between cultivars. The sterilized seeds were soaked in suspensions of *B. glumae* adjusted to  $OD_{520} = 0.04$  ( $\sim 10^7$  per mL) and held under vacuum for 1 min (Maeda *et al.* 2016). The inoculated seeds were dried overnight, sown in sterilized soil, and incubated in a growth chamber at 27°C with 80% humidity under a 14-h photoperiod. Disease symptoms were scored 7 days after sowing on a scale of 0 to 2, where 0 = no symptoms, 1 = sheaths with reddish-brown lesions (mild infection), and 2 = necrotic seedlings or seeds with no germination (severe infection). The BSR severity was calculated from these scores as:

$$\text{BSR severity (\%)} = (15 - (N_0 + N_1/2)) \times 100/15$$

where  $N_0$  = number of seedlings with score 0,  $N_1$  = number of seedlings with score 1, and 15 is the number of seeds per replication. There were three replications per inoculation. As a control, we germinated uninoculated seeds and confirmed that the average germination rate was >90%. The severity of BSR is increased by low germination vigor, which in turn can be caused by low seed quality. To control for the influence of seed quality on inoculation effectiveness, we inoculated seeds at 6 months and 1 year after har-

vest. All seeds were treated at 50°C for 5 days to break dormancy just before the inoculation test.

To analyze the correlation between panicle disease score (a measure of BGR resistance) and BSR score, we used the panicle disease scores of 43 major Japanese cultivars from our previous study (Mizobuchi *et al.* 2018). We used 45 cultivars (43 major Japanese cultivars, ‘Kele’, and RBG1-NIL) to analyze the relationships between panicle disease score and BSR score.

### Selection of standard cultivars for BSR resistance

We selected standard cultivars for BSR resistance to cover a range of resistance levels. In the Japan Plant Variety Registration System, cultivars are often classified for resistance on a scale of 0 (resistant) to 10 (susceptible). Therefore, we first converted “BSR severity” (a percentage) into a “BSR score” (a value from 0 to 10) and then converted the BSR score into a resistance level. BSR severity was divided by 10 to obtain the BSR score; thus, a score of 0 indicates a BSR severity of 0% (resistant) and a score of 10 indicates a BSR severity of 100% (susceptible). A disease score of <2 was classified as “very strong”,  $\geq 2$  to <4 as “strong”,  $\geq 4$  to <5 as “medium to strong”,  $\geq 5$  to <7 as “medium”,  $\geq 7$  to <9 as “weak to medium”, and  $\geq 9$  as “weak”.

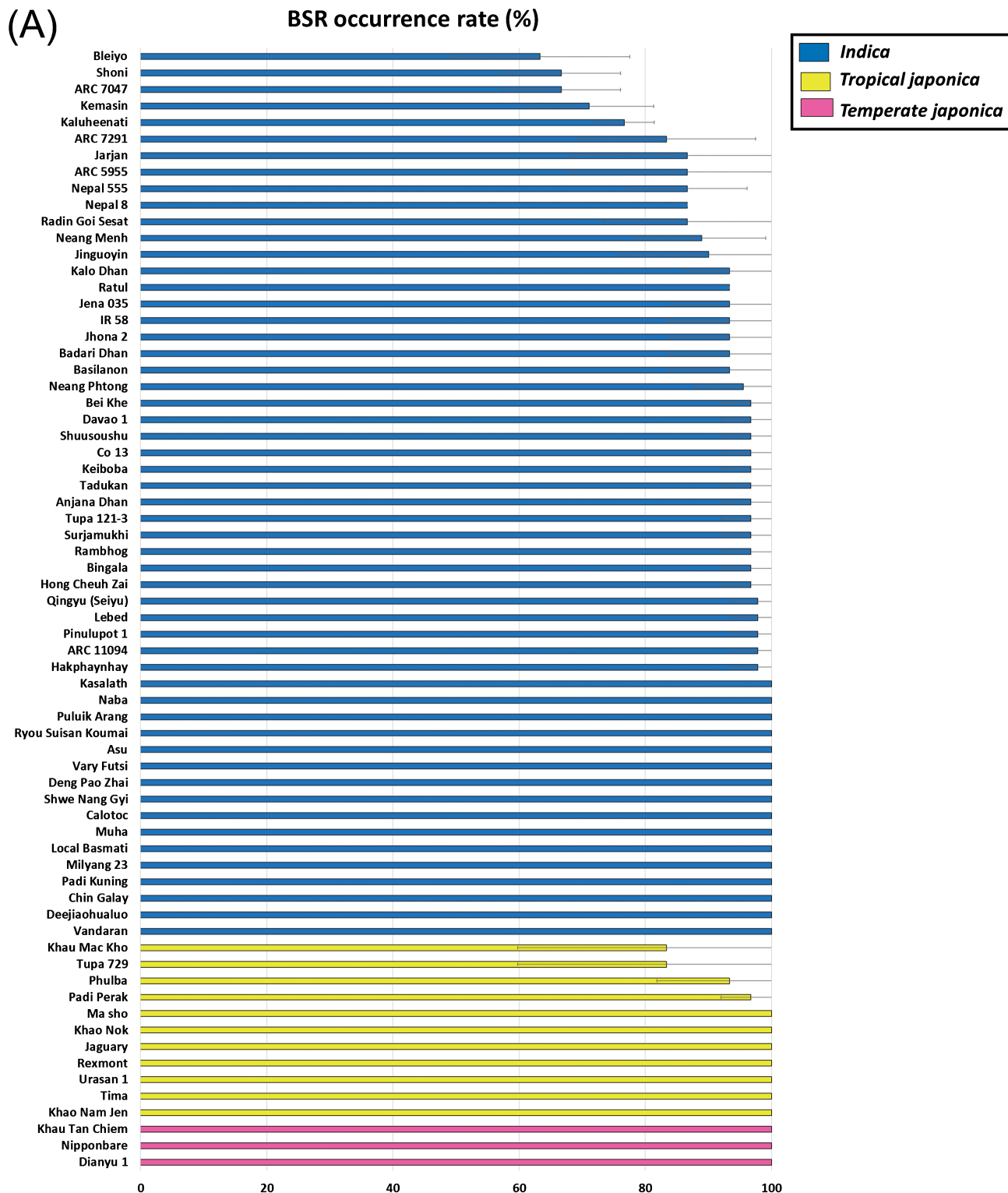
## Results

### Assessment of BSR occurrence rate

To identify resistant cultivars, we measured the BSR occurrence rate under high inoculation pressure. We evaluated the rate in 102 rice cultivars (**Fig. 1, Supplemental Table 1**), comprising 68 accessions from WRC (**Fig. 1A**) and 34 others (including 12 landraces from JRC) (**Fig. 1B**). Most seedlings of ‘Hitomebore’ had symptoms, and its BSR occurrence rate ranged from 83.3% to 100% (**Supplemental Table 1**). The occurrence rate in the tested lines ranged from 26.7% to 100% in *indica* cultivars, from 63.3% to 100% in *tropical japonica* cultivars, and from 51.1% to 100% in *temperate japonica* cultivars; the most resistant was ‘Nona Bokra’ (*indica*) (26.7%; **Fig. 1, Supplemental Table 1**). After ‘Nona Bokra’, ‘Bleiyo’ (63.3%), ‘Shoni’ (66.7%), ‘ARC 7047’ (66.7%), and ‘Kemasin’ (71.1%) were found to be the most resistant among *indica* cultivars and ‘LAC 23’ (63.3%) was found to be the most resistant among *tropical japonica* cultivars. Although almost all of the *temperate japonica* cultivars were highly susceptible, ‘Kujuu’, which was previously reported to be resistant to BSR (Hirashima and Wakimoto 1983), was found to be the most resistant (51.1%). These results indicate wide variation in BSR resistance among cultivars.

### Assessment of BSR resistance

Next, we evaluated 43 major Japanese cultivars for BSR resistance, four of which (Hitomebore, Koshihikari, Nipponbare, and Sasanishiki) were among the 102 cultivars



**Fig. 1.** Bacterial seedling rot (BSR) occurrence rates of 102 cultivars. (A) Cultivars from WRC (World Rice Collection of NARO). (B) (1) Landraces from JRC (Japanese Rice Collection of NARO), (2) cultivars reported as BSR-resistant or bacterial grain rot (BGR)-resistant, and (3) cultivars used to develop mapping populations. Error bars indicate SD.

assessed for BSR occurrence rate. We also selected 9 relatively resistant cultivars (Aikoku, ARC 7047, Bleiyo, Kele, Kemasin, Kujuu, LAC 23, Nona Bokra, and Shoni) from

the BSR occurrence test for comparison. We measured the levels of BSR resistance of these 52 cultivars and of *RBGI-NIL*, which has the *RBGI* genomic region from ‘Nona



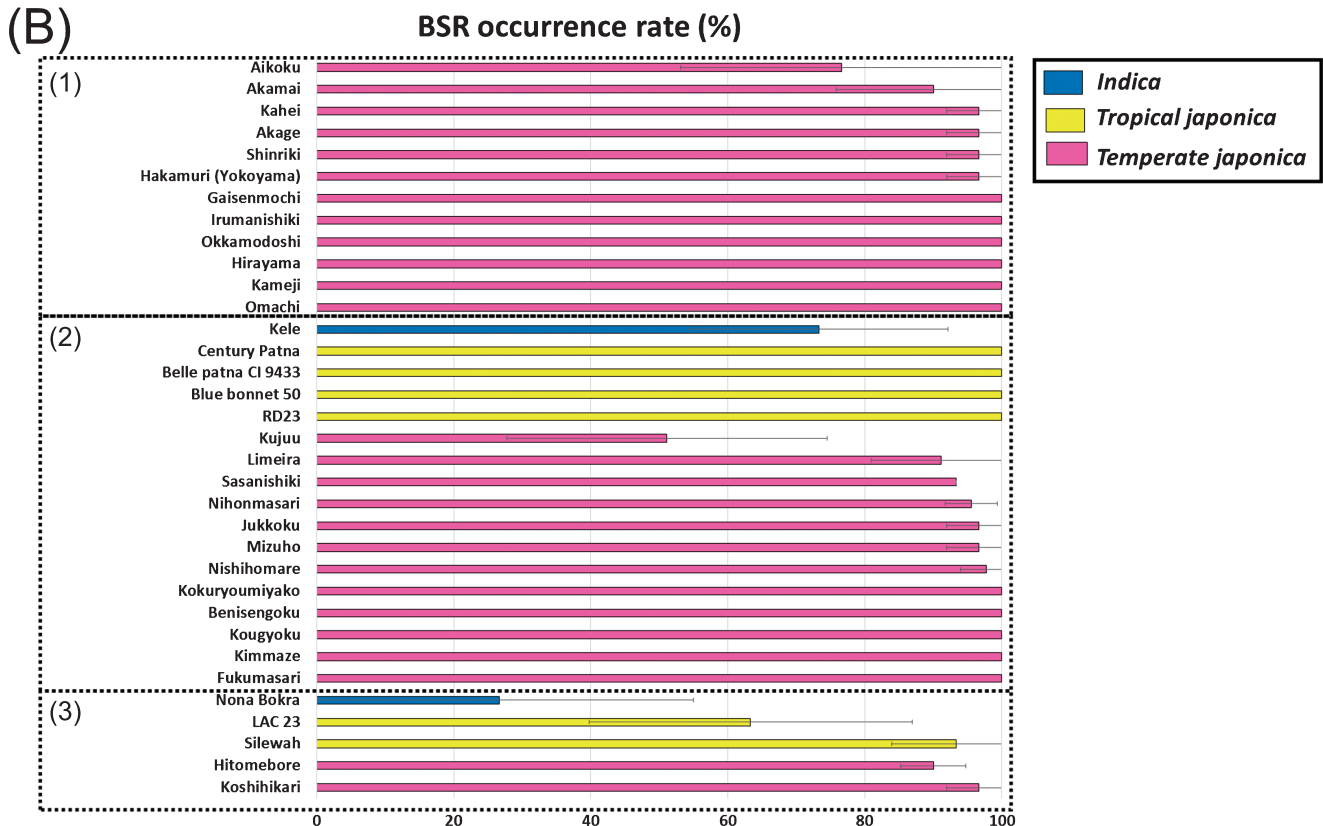


Fig. 1. (continued)

Bokra' in a 'Koshihikari' genetic background, at 6 months and 1 year after harvest to control for the influence of seed quality on inoculation effectiveness.

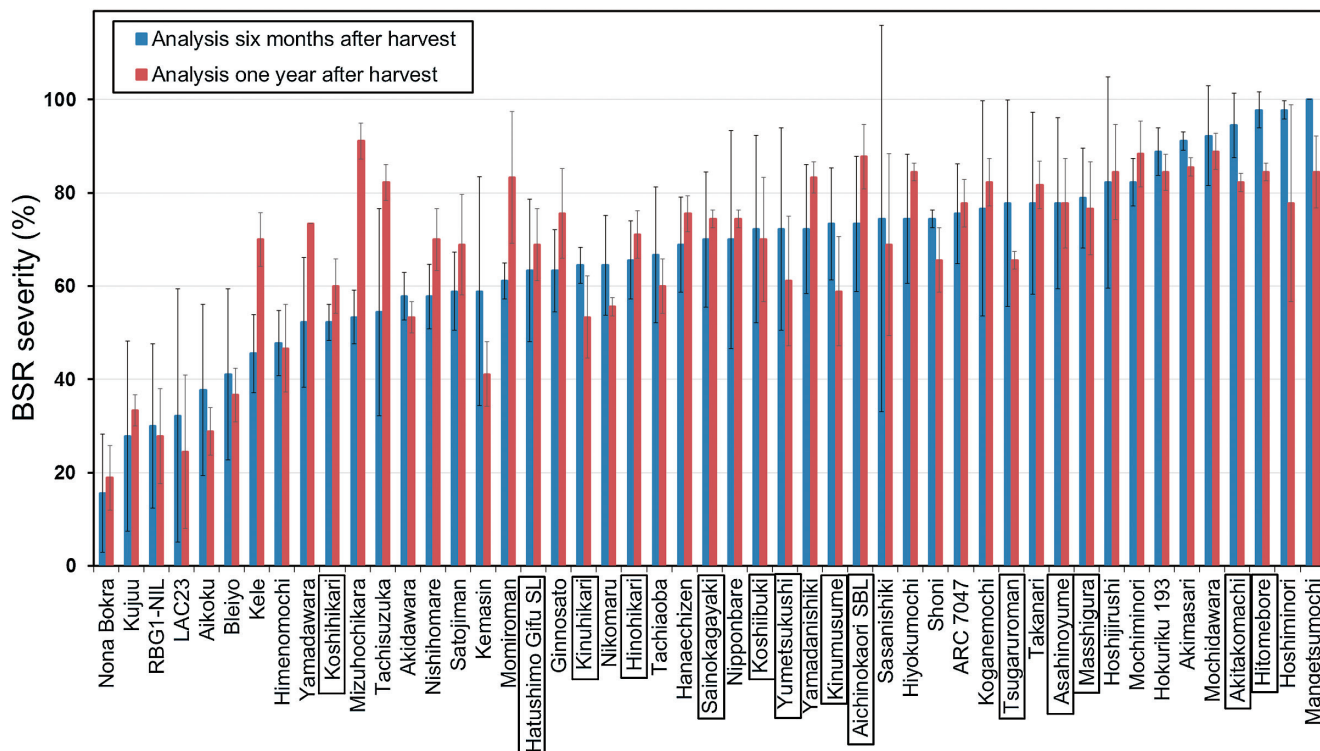
The BSR severity assessed at 6 months after harvest ranged from 15.6% ('Nona Bokra') to 100% ('Mangetsu-mochi') (Fig. 2, Supplemental Table 2); that assessed at 1 year after harvest ranged from 18.9% ('Nona Bokra') to 91.1% ('Mizuhochikara') (Fig. 2, Supplemental Table 2). Although the BSR severity of several cultivars was different at 1 year than at 6 months (Fig. 2), the correlation coefficient was significant at the 1% level ( $r=0.778$ ) (Supplemental Fig. 1). Therefore, the assessment method appears to be reliable for evaluating BSR resistance. Because the data from the 6-month and 1-year assessments were generally consistent, we used the average BSR severity from the two experiments to determine the BSR score (=BSR severity  $\div$  10) for each cultivar (Supplemental Table 2).

Among the 13 cultivars analyzed for both BSR occurrence (Fig. 1) and BSR severity (Fig. 2), some showed different tendencies in these two measurements, presumably because of different inoculation concentrations and evaluation methods. 'Aikoku' had a relatively high BSR occurrence rate (Fig. 1) but low disease severity, comparable to that of 'Kujuu' (Fig. 2); similarly, 'Koshihikari' had high BSR occurrence rates (Fig. 1) but lower disease severity (Fig. 2). However, other cultivars had the same tendencies

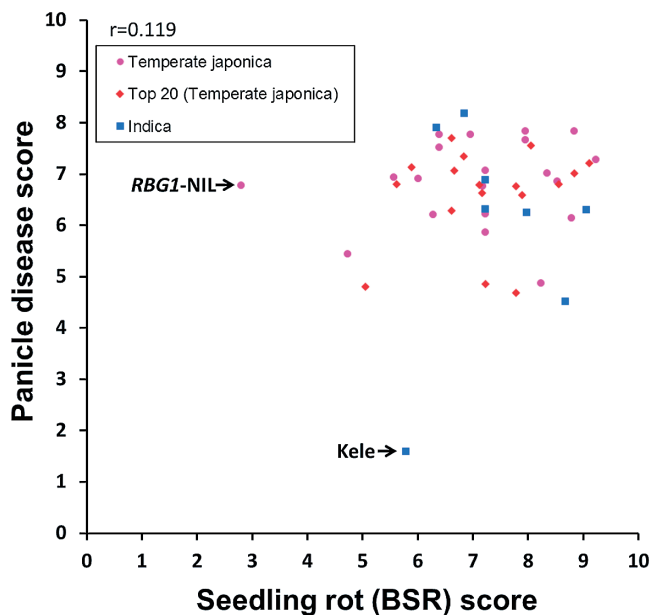
in BSR occurrence rate (Fig. 1) and BSR disease severity (Fig. 2). Across the entire set of cultivars analyzed for disease severity, the BSR scores ranged from 1.7 ('Nona Bokra') to 9.2 ('Mangetsu-mochi') (Fig. 2, Supplemental Table 2). Most of the major Japanese cultivars, including 'Koshihikari', the leading cultivar in Japan, had scores of  $\geq 5$  to  $< 7$  ("medium") or  $\geq 7$  to  $< 9$  ("weak to medium") (Fig. 2, Supplemental Table 2). 'Nona Bokra' (*indica*) was classified as having "very strong" resistance. 'Bleiyo' (*indica*), 'LAC 23' (*tropical japonica*), and 'Kujuu' and 'Aikoku' (*temperate japonica*) had "strong" resistance (Fig. 2, Supplemental Table 2). The BSR score of *RBG1-NIL* was 2.9 ("strong"), which was more resistant than any of the *temperate japonica* cultivars but less resistant than 'Nona Bokra', the donor of *RBG1* (Supplemental Table 2).

We also evaluated 43 major Japanese rice cultivars, 'Kele' and *RBG1-NIL* previously analyzed for BGR resistance (Mizobuchi *et al.* 2018) for BSR resistance. The correlation between the scores for BSR and BGR was low (Fig. 3).

*RBG2-NIL*, produced by introducing the genomic region containing *RBG2* (Mizobuchi *et al.* 2015) for BGR resistance from 'Kele' into 'Hitomebore', was still under development when the screening began, but we compared it with 'Hitomebore' later. Although *RBG2-NIL* was susceptible to BSR, it was less susceptible than 'Hitomebore' (Supplemental Fig. 2).



**Fig. 2.** BSR severity of 49 cultivars. Boxes indicate “Top 20” cultivars. Four cultivars from the full analysis were omitted from this figure because we were not allowed to publicize their names. Error bars indicate SD.



**Fig. 3.** Correlation between panicle disease score and BSR score. We assessed 43 major Japanese rice cultivars previously evaluated for BGR resistance (Mizobuchi *et al.* 2018), ‘Kele’ (BGR resistant), and *RBG1-NIL* (containing a BSR-resistance QTL).

### Selection of standard cultivars for BSR resistance

On the basis of the results from BSR assessment (Fig. 2, Supplemental Table 2), we selected standard cultivars for BSR resistance assessment according to both resistance

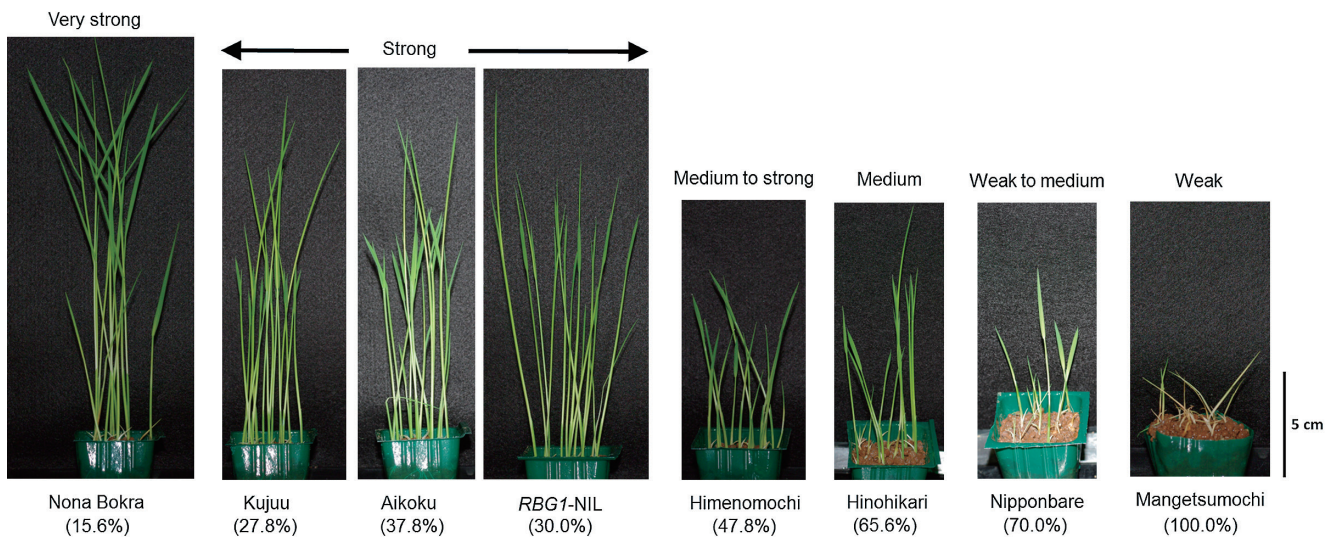
level and resistance stability (i.e., consistency across repeated assessments) (Table 1). Because the BSR severity of several cultivars was higher in the second experiment (at 1 year) than in the first (at 6 months; Fig. 2), those cultivars were eliminated from consideration as standard cultivars. To represent the “very strong” resistance class, we selected ‘Nona Bokra’. Following seed inoculation, most seedlings of ‘Nona Bokra’ looked normal, and only a few had sheaths with brown lesions. To represent the “strong” class, we selected ‘LAC 23’, ‘Kujuu’, and ‘Aikoku’. Among these cultivars, about half of the seedlings from inoculated seeds had no symptoms and the other half had sheaths with brown lesions. To represent “medium to strong” cultivars, we selected ‘Himenomochi’. Most seedlings from inoculated seeds of ‘Himenomochi’ had sheaths with brown lesions. As “medium” cultivars, we selected ‘Koshihikari’, ‘Akidawara’, and ‘Hinohikari’. About two-thirds of the seedlings from inoculated seeds of these cultivars had sheaths with brown lesions (mild infection) and the others were necrotic (severe infection). As “weak to medium” cultivars, we selected ‘Takanari’ and ‘Nipponbare’. Most seedlings from inoculated seeds of these cultivars had severe phenotypes such as whitening of leaf blades and sheaths. As “weak” cultivars, we selected ‘Hitomebore’ and ‘Mangetsumochi’. Almost all of the seedlings from inoculated seeds of these cultivars were necrotic, and some seeds did not germinate at all. Typical responses of these cultivars are shown in Fig. 4.

**Table 1.** Standard cultivars for BSR resistance, classified according to resistance level

Category	Resistance level											
	Very strong		Strong		Medium to strong		Medium		Weak to medium		Weak	
	Cultivar name	Score <sup>a</sup>	Cultivar name	Score <sup>a</sup>	Cultivar name	Score <sup>a</sup>	Cultivar name	Score <sup>a</sup>	Cultivar name	Score <sup>a</sup>	Cultivar name	Score <sup>a</sup>
<i>Indica</i>	Nona Bokra	1.7							Takanari	8.0		
<i>Tropical japonica</i>			LAC 23	2.8								
<i>Temperate japonica</i>			Kujuu	3.1	Himenomochi	4.7	Koshihikari	5.6	Nipponbare	7.2	Hitomebore	9.1
			Aikoku	3.3			Akidawara	5.6			Mangetsumochi	9.2
							Hinohikari	6.8				

<sup>a</sup> Each score was calculated by using the average BSR severity from analyses 6 months and 1 year after harvest (Supplemental Table 2). Average BSR severity was divided by 10 to obtain the BSR score. A score of 0 indicates an average BSR severity of 0% (resistant); a score of 10 indicates an average BSR severity of 100% (susceptible).

The inoculation method is described under “Assessment of BSR resistance” in “Materials and Methods”.



**Fig. 4.** Differences in resistance to BSR among the set of standard cultivars selected in this study (Table 1). Photos show results from the first experiment (seeds inoculated 6 months after harvest), and the BSR severity of each cultivar from that experiment is shown in parentheses.

## Discussion

Because BSR and BGR caused by *B. glumae* are two of the most common diseases of rice in Japan, we previously evaluated major Japanese rice cultivars for BGR resistance and selected a set of standard cultivars for use in future BGR resistance assessments (Mizobuchi *et al.* 2018). Here we evaluated major rice cultivars for BSR resistance and selected a set of Japanese standard cultivars for that trait. The evaluation was done in two steps: the first measured BSR occurrence rate in over 100 Japanese and world cultivars under high inoculation pressure, the second measured BSR resistance in primarily Japanese cultivars under lower inoculation pressure. Although a high inoculum concentration ( $10^8$  per mL) was suitable for identifying resistant cultivars from WRC and other sources, it was necessary to lower the concentration (to  $10^7$  per mL) to detect small differences in resistance between major Japanese cultivars. We measured the BSR occurrence rate in 102 rice cultivars (Fig. 1, Supplemental Table 1), comprising 68 accessions

from WRC (Fig. 1A) and 34 others (Fig. 1B). Among the latter 34 accessions, 12 were landraces from JRC, 17 were cultivars previously reported as BSR- or BGR-resistant (Goto and Watanabe 1975, Hirashima and Wakimoto 1983, Mizobuchi *et al.* 2013a, Prabhu and Bedendo 1988, Wasano and Okuda 1994, Yokoyama and Okuhara 1987), and 5 had been used to develop mapping populations (Abe *et al.* 2013, Fukuoka *et al.* 2010, Takai *et al.* 2007) (Supplemental Table 1). Among the 17 cultivars with reported resistance, ‘Kujuu’ was previously reported to be resistant to BSR (Hirashima and Wakimoto 1983) and the other 16 were previously reported to be resistant to BGR (Goto and Watanabe 1975, Mizobuchi *et al.* 2013a, Prabhu and Bedendo 1988, Wasano and Okuda 1994, Yokoyama and Okuhara 1987). The 102 cultivars consisted of 57 cultivars of *indica*, 17 of *tropical japonica*, and 28 of *temperate japonica* (Supplemental Table 1).

Assessment of BSR occurrence rate is useful for finding resistant cultivars, but is inappropriate for detecting small differences in resistance between cultivars. Thus, we used BSR severity when we assessed major Japanese cultivars.



In the Japan Plant Variety Registration System, however, cultivars are often classified by resistance score, so we first converted “BSR severity” (a percentage) into “BSR score” (a value from 0 to 10) and then converted the BSR score into a resistance level. ‘Nona Bokra’ (*indica*) showed “very strong” resistance. ‘Bleiyo’ (*indica*), ‘LAC 23’ (*tropical japonica*), and ‘Kujuu’ and ‘Aikoku’ (*temperate japonica*) showed “strong” resistance (Fig. 2, Supplemental Table 2). Although no *temperate japonica* cultivars were scored as “strong” or “medium to strong” for BGR resistance in our earlier study (Mizobuchi *et al.* 2018), two *temperate japonica* cultivars, ‘Kujuu’ and ‘Aikoku’, showed BSR resistance in the present study and so would be useful donors for BSR resistance for *temperate japonica* cultivars. ‘Kujuu’ was previously reported to be resistant to BSR by screening inoculation of sterilized seeds (Hirashima and Wakimoto 1983); thus, our results confirm these previous findings. Because ‘Aikoku’ is one of the ancestors of ‘Kujuu’, we hypothesize that a common genetic mechanism exists in both. Haplotype analysis based on next-generation sequencing (NGS) can be conducted in rice (Ogawa *et al.* 2018). By comparing genetic data (NGS data) and phenotypic data (BSR resistance scores) between ‘Aikoku’ and other ancestors of ‘Kujuu’, we will clarify the genetic basis of resistance in ‘Aikoku’ and ‘Kujuu’.

On the other hand, our results indicate that most of the major Japanese cultivars, including ‘Koshihikari’, and other “Top 20” cultivars have “medium” or “weak to medium” BSR resistance; none were scored as “very strong”, “strong” or “medium to strong” (Fig. 2, Supplemental Table 2). Although oxolinic acid was developed as an agricultural chemical for BSR control in Japan (Hikichi 1993a, 1993b, Hikichi *et al.* 1989), many strains of *B. glumae* tolerant to oxolinic acid have been found in several prefectures (Fukushi *et al.* 2000, Hori *et al.* 2004, Minagawa and Yamada 2008, Morikawa *et al.* 1997, Ohtani and Takeuchi 2013, Yamashita *et al.* 1998). Therefore, the need for cultivars carrying genes for resistance to BSR is increasing as a means to ensure sustainable rice production. Thus, it will be necessary to introduce QTLs from resistant cultivars into Japanese *temperate japonica* cultivars to develop BSR-resistant cultivars for use in Japan.

In a previous study, we finely mapped a QTL (*RBG1*) for BSR resistance from ‘Nona Bokra’, a resistant traditional lowland cultivar (*indica*) that originated in India (Mizobuchi *et al.* 2013b), and developed a near-isogenic line (*RBG1-NIL*) by introducing the genomic region containing *RBG1* into ‘Koshihikari’. The score of *RBG1-NIL* was 2.9, which is classified as “strong”, although the resistance level was less than that of ‘Nona Bokra’ (Figs. 2, 4, Supplemental Table 2). Therefore, we expect *RBG1-NIL* to be released as the first BSR-resistant Japanese *temperate japonica* cultivar. *RBG2*, a QTL for BGR resistance from Kele, also confers resistance to BSR (Supplemental Fig. 2), but its effect is minor. Therefore, rice breeders will need to combine *RBG1*, *RBG2*, and additional QTLs to improve stable

resistance to both BSR and BGR. As we succeeded in identifying other BSR-resistant cultivars besides ‘Nona Bokra’, it might be possible to identify other new QTLs that will be useful for gene pyramiding to improve BSR resistance. Although many disease resistance genes have been cloned and characterized in rice (Singh *et al.* 2018), no resistance genes associated with BSR or BGR have yet been identified. The cloning of *RBG1* and *RBG2* is under way to clarify their genetic mechanisms.

Major rice cultivars that were previously analyzed for BGR resistance (Mizobuchi *et al.* 2018) were evaluated for BSR resistance. Although ‘Kele’ is a promising genetic resource for resistance to both BSR and BGR, the overall correlation between the BSR and BGR scores was low (Fig. 3), suggesting that different factors are associated with resistance to each. Our results indicate that it is necessary to introduce QTLs from different sources to develop cultivars resistant to both BSR and BGR.

We conducted inoculation tests for assessment of BSR resistance at two different times and found a strong correlation between the data from the first and second experiments (Fig. 2, Supplemental Fig. 1). Although the level of resistance in the two experiments tended to be the same for most cultivars, several cultivars were more susceptible in the second experiment than in the first, and vice versa (Fig. 2, Supplemental Fig. 1). This indicates that the characteristics of seed quality at different times after harvest differed between cultivars. Thus, it is necessary to compare the BSR severity between seeds harvested in the same year and to conduct the experiments at more than one time point after harvest.

This study was based on results using only one *B. glumae* strain, MAFF 301682. The resistance of the tested cultivars may differ when challenged with different strains. Further, because this whole study was conducted in Tsukuba, it is important that the standard cultivars selected here (Table 1) be evaluated in other areas.

### Author Contribution Statement

RM carried out resistance analysis and wrote the manuscript; SF helped to draft the manuscript; CT helped with resistance analysis; ST provided advice on the method of resistance analysis; HS coordinated the study design and helped to draft the manuscript. All authors read and approved the final manuscript.

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