Breeding Science 66: 396–406 (2016)
doi:10.1270/jsbbs.15157

Research Paper

# Overexpression of *BSR1* confers broad-spectrum resistance against two bacterial diseases and two major fungal diseases in rice

**Satoru Maeda[1)], Nagao Hayashi[1)], Takahide Sasaya[2,3)] and Masaki Mori*[1)]**

[1)] *Institute of Agrobiological Sciences, NARO (NIAS)*, Tsukuba, Ibaraki 305-8602, Japan
[2)] *NARO Agricultural Research Center (NARC)*, Tsukuba, Ibaraki 305-8666, Japan
[3)] *Present address: NARO*, Tsukuba, Ibaraki 305-8517, Japan

Broad-spectrum disease resistance against two or more types of pathogen species is desirable for crop improvement. In rice, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the causal bacteria of rice leaf blight, and *Magnaporthe oryzae*, the fungal pathogen causing rice blast, are two of the most devastating pathogens. We identified the rice *BROAD-SPECTRUM RESISTANCE 1* (*BSR1*) gene for a BIK1-like receptor-like cytoplasmic kinase using the FOX hunting system, and demonstrated that *BSR1*-overexpressing (OX) rice showed strong resistance to the bacterial pathogen, *Xoo* and the fungal pathogen, *M. oryzae*. Here, we report that *BSR1*-OX rice showed extended resistance against two other different races of *Xoo*, and to at least one other race of *M. oryzae*. In addition, the rice showed resistance to another bacterial species, *Burkholderia glumae*, which causes bacterial seedling rot and bacterial grain rot, and to *Cochliobolus miyabeanus*, another fungal species causing brown spot. Furthermore, *BSR1*-OX rice showed slight resistance to rice stripe disease, a major viral disease caused by rice stripe virus. Thus, we demonstrated that *BSR1*-OX rice shows remarkable broad-spectrum resistance to at least two major bacterial species and two major fungal species, and slight resistance to one viral pathogen.

**Key Words:** rice, receptor-like cytoplasmic kinase, broad-spectrum disease resistance, *Magnaporthe oryzae*, *Xanthomonas oryzae*, *Cochliobolus miyabeanus*, *Burkholderia glumae*.

## Introduction

In the natural environment, plants encounter many species of pathogenic microorganisms, such as fungi, bacteria and viruses. The damage caused by microbial diseases is one of the most important limiting factors for crop production. To solve this problem, improvement of host resistance against these pathogens is the most economical and environmentally friendly approach. Rice (*Oryza sativa* L.) is one of the most important food crops and is a staple food for approximately 50% of the world's population (Liu *et al.* 2014). Moreover, it is a model plant of monocotyledonous species. Bacterial leaf blight caused by bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), blast by fungus *Magnaporthe oryzae*, brown (leaf) spot by fungus *Cochliobolus miyabeanus*, and stripe mosaic caused by viral pathogen rice stripe virus are the major rice diseases worldwide and result in serious losses of rice production.

Communicated by M. Ohshima
Received December 1, 2015. Accepted February 8, 2016.
First Published Online in J-STAGE on May 20, 2016.
*Corresponding author (e-mail: morimasa@affrc.go.jp)

To breed blast-resistant rice, efforts have aimed to introduce the resistance (*R*) genes into susceptible cultivars. The *R* gene is a key component of disease resistance to a particular pathogen and is often associated with a hypersensitive response (HR) (Flor 1971). In most cases, the resistant cultivars with *R* genes remain effective for only a few years in agricultural production (Dean *et al.* 2005) because new biotypes of the pathogen that can overcome the *R* gene often appear after release of the resistance cultivar. By contrast, although large numbers of quantitative trait loci (QTLs) (or quantitative genes) for bacterial leaf blight or blast resistance have been identified, these sources have not been used effectively in rice improvement because the genetic control of quantitative resistance is complex. Therefore, breeding for cultivars that exhibit broad-spectrum and durable disease resistance is a top priority in rice improvement programs around the world.

In addition to bacterial leaf blight and blast, there are several other important diseases in rice, such as brown spot and bacterial seedling rot. Brown spot disease is caused by the fungus *C. miyabeanus*, a representative necrotrophic pathogen, and is one of the most prevalent diseases in all rice-growing areas. *C. miyabeanus* infects plant tissues such

as leaves and spikelets in all development stages. Fungicides, such as iprodione and propiconazole, are effective means to manage this disease (Moletti *et al.* 1997). However, the use of resistant varieties would be preferable because fungicides are expensive and not environmentally friendly. Meanwhile, rice cultivar 'Tadukan' offers quantitative resistance to brown spot disease. However, no major genes conferring immunity to this disease have been identified, although three QTLs for disease resistance have been identified (Sato *et al.* 2008). So far, genetic studies of resistance to brown spot disease have been superficial.

Bacterial seedling rot and bacterial grain rot (bacterial panicle blight) in rice are caused by bacterial pathogen *Burkholderia glumae*, which is also a necrotrophic pathogen (Iwai *et al.* 2002, Mizobuchi *et al.* 2013b). Recently, these diseases have become an increasingly serious problem in global rice production because of global warming and climate change; *B. glumae* prefers high temperature and humidity (Ham *et al.* 2011). Some studies reported partially resistant varieties for these diseases and several QTLs for resistance to bacterial seedling rot and bacterial grain rot have been identified (Mizobuchi *et al.* 2013a, 2013b, 2015, Pinson *et al.* 2010). However, these resistances are not strong. Meanwhile, Iwai *et al.* (2002) reported that transgenic rice lines overproducing *Asthi1*, an oat leaf thionin gene, showed enhanced resistance to *B. glumae*. However, it has not been applied to actual breeding.

Rice stripe disease, caused by rice stripe virus (RSV), is one of the major viral diseases in East Asia. The majority of *japonica* cultivars in East Asia are highly susceptible to RSV (Wang *et al.* 2014) although a few rice cultivars/lines that show resistance to RSV have been described (Noda *et al.* 1991, Zhang *et al.* 2011).

Breeding crops with broad-spectrum disease resistance using genetic resources is one of the principal goals of crop improvement. However, only a few genes have been identified as genetic resources for broad-spectrum disease resistance in rice. Hence, the transgenic approach could be a viable alternative. In recent years, several gain-of-function transgenic mutant populations have been developed in rice (Hsing *et al.* 2007, Jeong *et al.* 2002, Mori *et al.* 2007, Nakamura *et al.* 2007). Meanwhile, Kondou *et al.* (2009) produced more than 20,000 independent *Arabidopsis* transgenic lines overexpressing rice full-length cDNAs (rice-FOX *Arabidopsis* lines) to enable high-throughput screening for rice genes. We performed screening for pathogen resistance using these lines (Dubouzet *et al.* 2011). As a result, we identified several rice genes conferring resistance to both bacterial *Pseudomonas syringae* pv. *tomato* DC3000 and fungal *Colletotrichum higginsianum* in *Arabidopsis*. One of the genes named *BROAD-SPECTRUM RESISTANCE1* (*BSR1*), encoding a receptor-like cytoplasmic kinase, conferred resistance to *Xoo* and *M. oryzae* when overexpressed in rice (Dubouzet *et al.* 2011).

In this paper, we report that overexpression of *BSR1* not only conferred non-race-specific resistance to *Xoo* and *M. oryzae*, but also extended resistance to *B. glumae* and *C. miyabeanus*. Moreover, overexpression of *BSR1* is likely to confer partial resistance to RSV.

## Materials and Methods

### *Plant materials*

Rice (*Oryza sativa* L.) wild-type (WT) cultivar 'Nipponbare', *Xoo*-resistant cultivar 'Asominori', RSV-resistant cultivar 'Sainokagayaki' and two transgenic plant lines (*BSR1*-OX-5 and -9) were grown under greenhouse conditions at 27°C to 30°C. *BSR1*-OX-5 and *BSR1*-OX-9 correspond to the previously reported *AK070024*:OX-5 and *AK070024*:OX-9 (Dubouzet *et al.* 2011), respectively.

For disease resistance tests, except for *B. glumae*, dehusked seeds were surface sterilized, sown on one-half-strength MS medium (Wako Pure Chemicals, Osaka, Japan), containing 3% (w/v) sucrose and 0.4% (w/v) Gelrite (Wako Pure Chemicals), in Agripots and grown in the growth chamber at 28°C in the dark for 3 days, then at 25°C under long-day conditions (16 h light [60–70 μmol $m^{-2}$ $s^{-1}$]/8 h dark) for 4–7 days. For transgenic seeds, Hygromycin B (30–50 μg/ml; Wako Pure Chemicals) was added to the medium. WT seedlings and hygromycin-resistant transgenic seedlings were transplanted into soil (Bonsol No. 2, Sumitomo Kagaku Kougyo, Osaka, Japan) and used for disease resistance tests.

### *Pathogens and pathogen cultures*

The bacterial isolates used in this study were T7147 (MAFF311019, race II) and T7133 (MAFF311020, race III) of *Xoo* and AZ8204 (MAFF301682) of *B. glumae*, and the fungal isolates were Hoku1 (MAFF101512, race 007.0) of *M. oryzae* and H11-42-1 of *C. miyabeanus*.

Culture procedures for the various pathogens for inoculum were as follows. *Xoo* were cultured on PSA agar plates (1% proteose peptone, 1% sucrose, and 1.5% bacto agar) for 2 days at 28°C under dark conditions. *B. glumae* were cultured on King B agar plate (2% proteose peptone, 0.15% $K_2HPO_4$, 0.15% $MgSO_4$, 1% glycerin and 1.5% agar, Eiken chemical, Tochigi, Japan) at 28°C for 2 days under dark conditions. *M. oryzae* was grown on oatmeal agar plates (3% oatmeal, 0.5% sucrose, and 1.6% bacto agar) at 25°C in the dark for 10 days, then under continuous illumination for 4 days to induce sporulation. *C. miyabeanus* was grown on V8 agar plates (20% V8 juice (Campbell soup company, Camden, NJ, USA), 0.3% $CaCo_3$, and 1.5% bacto agar) at 25°C in the dark for 5 to 6 days, and then under a 12/12 h light/dark regime for 3 to 4 days to induce sporulation. The cultured pathogens were scraped and used to produce inocula.

### *Expression analysis of* BSR1 *by quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR)*

Total RNA was isolated from rice leaves using Isogen (Wako Pure Chemicals) followed by further purification

with the RNeasy mini kit (Qiagen, Valencia, CA, USA). First-strand cDNAs were synthesized from equal amounts of total RNA using a ReverTra Ace qPCR RT Master Mix with gDNA Remover Kit (Toyobo, Osaka, Japan) in a total volume of 10 µl, as described by the manufacturer. qRT-PCR was performed with the Thermal Cycler Dice TP800 system (Takara, Tokyo, Japan) using a Kapa SYBR FAST qPCR kit (Kapa Biosystems, Cape Town, South Africa) as described by the manufacturer. The primers used for qPCR were as follows: *BSR1* 5′-AGGTGAGGTTGCACTCTGCT-3′ and 5′-CCAAGAATCCACCAACTCGT-3′ as described Dubouzet *et al.* (2011); those for *Rubq1* were 5′-GGAGC TGCTGCTGTTCTAGG-3′ and 5′-TTCAGACACCATCAA ACCAGA-3′ as an internal control, as described Jiang *et al.* (2010). Transcript levels of *BSR1* were normalized to the endogenous rice reference gene (*Rubq1*).

### *Test for resistance to* Xoo

Rice seedlings transplanted in soil were grown in a growth chamber until the 6–8-leaf stage at 25°C under long-day conditions (16 h light/8 h dark) and then used for evaluation. The suspensions of *Xoo* (isolate T7147 or T7133) for inocula were adjusted to $OD_{600}$ = 0.3 with sterile water. The top leaf blades of the tested plants were cut with scissors pre-wetted with inoculum at about 5 cm from the tip, and the cut ends (about 5 mm from the end) were dipped in inoculum for 10 s. The disease symptoms (lesion length) of inoculated plants were assessed 2 weeks after inoculation, as described previously (Dubouzet *et al.* 2011).

### *Test for resistance to* M. oryzae

Rice seedlings transplanted in soil were grown in a greenhouse until the four-leaf stage at 28°C under a natural photoperiod. The plants were inoculated by spraying a spore suspension of *M. oryzae* (isolate Hoku1 (MAFF101512, race 007.0)). The detailed procedure for producing spore suspension is as follows: The mycelia of *M. oryzae* were scraped and the gel surface was flooded with sterile water containing 0.01% Tween 20. The suspension was filtered through a Kimwipe, and the resulted spore suspension was collected. The spore suspension was adjusted to a concentration of $6.7 \times 10^5$ spores/ml and used for inoculation. After inoculation, the plants were placed in a dark chamber at 26°C and 100% humidity for 20 h, and then further cultured in the greenhouse. Evaluation of resistance was based on the total number of compatible lesions that appeared on the 3rd and 4th leaf blades of each plant 5 days after inoculation.

### *Evaluation of bacterial pathogen* B. glumae *resistance*

WT 'Nipponbare' rice seeds and $T_{3–5}$ seeds of *BSR1*-OX-5 and -9 were sterilized by soaking in 70% ethanol for 30 s and Antiformin (available chlorine 5%) for 20 min. The seeds were then rinsed with sterilized water. The sterilized seeds were soaked in sterilized water at 28°C for 2 to 3 days in the dark and pre-germinated to 1–2 mm of sprout. The pre-germinated seeds were soaked in suspensions of *B. glumae* adjusted to $OD_{520}$ = 0.004 and held under a vacuum for 1 min. The inoculated seeds were dried and sown in sterilized soil (Bonsol No. 2, Sumitomo Kagaku Kougyo). The inoculated seeds were incubated in a growth chamber at 28°C with 100% humidity under a 14-h photoperiod. Plant phenotypes were classified as 'healthy' or 'diseased' at 7–10 days after inoculation and the percentage of healthy plants among the total seeds used for inoculation was calculated as the survival ratio.

### *Evaluation of fungal pathogen* C. miyabeanus *resistance*

Rice seedlings transplanted in soil were grown in a greenhouse until the six-leaf stage at 28°C under a natural photoperiod. The inoculation method of *C. miyabeanus* was the same as that of *M. oryzae*, except as follows. The concentration of the spore suspension was adjusted to $10^4$ spores/ml. Evaluation of resistance was based on the total number of compatible lesions that appeared on the 5th and 6th leaf blades of each plant 5 days after inoculation.

### *Assessment of resistance to RSV*

*BSR1*-OX seedlings selected by Hygromycin resistance (30–50 µg/ml), WT and cv. 'Sainokagayaki' seedlings were transplanted into soil at about 10 seedlings per pot, and grown in a greenhouse until the two-leaf stage at 28°C under a natural photoperiod. The rice seedlings were exposed to approximately 10 viruliferous small brown planthoppers (*Laodelphax striatellus* Fallén) per plant in an inoculation cage for 1 day to inoculate RSV, as described previously (Satoh *et al.* 2010). After the inoculation period, the insects were killed with insecticide and the seedlings were transferred to the same greenhouse. The virus infection was evaluated by enzyme-linked immunosorbent assay (ELISA) using antiserum against RSV nucleocapsid protein, as described previously (Shimizu *et al.* 2011). The pieces (about 1 cm) of leaf sheath plus stem tissue from inoculated seedlings were harvested for ELISA at 16 days after inoculation. Resistance to RSV was calculated by the ratio of diseased seedlings detected by ELISA among all inoculated seedlings.

## Results

### *Transcript level of* BSR1 *in* BSR1-*OX rice lines*

The cDNA of *BSR1* was inserted downstream of the constitutive maize *ubiquitin* promoter (**Fig. 1A**), and the construct was used to generate transgenic rice lines overexpressing *BSR1*, as described previously (Dubouzet *et al.* 2011). The resulting two transgenic lines, *BSR1*-OX-5 (former name, *AK070024*:OX-5) and -9, were used for various disease resistance tests. To gain sufficient seeds for the disease resistance tests, the two transgenic lines were subjected to acceleration of advanced generations. To confirm overexpression of *BSR1*, we examined the transcript level of *BSR1* by qRT-PCR in $T_{3–4}$ generations of the *BSR1*-OX lines (**Fig. 1B**). Transcript levels of *BSR1*-OX-5 and -9 lines were 159- and 130-fold higher than that of 'Nipponbare' (WT),

respectively. Thereafter, we used the plants of $T_{3–5}$ lines for various disease resistance tests.

### *Overexpression of* BSR1 *confers resistance to multiple races of* Xoo *and* M. oryzae *in rice*

We have reported that *BSR1*-OX rice shows strong resistance to isolate T7174 (race I) of *Xoo*, a bacterial pathogen for rice bacterial leaf blight, and to isolate Kyu89-246 (race 003.0) of *M. oryzae*, a fungal pathogen for rice blast (Dubouzet *et al.* 2011). Hence, it would be plausible that *BSR1* also confers resistance to other races of *Xoo* and *M. oryzae*. First, we examined whether *BSR1-OX* rice extended resistance to isolates T7147 (race II) and T7133 (race III) of *Xoo*. The WT and the resistant control, cv. 'Asominori',

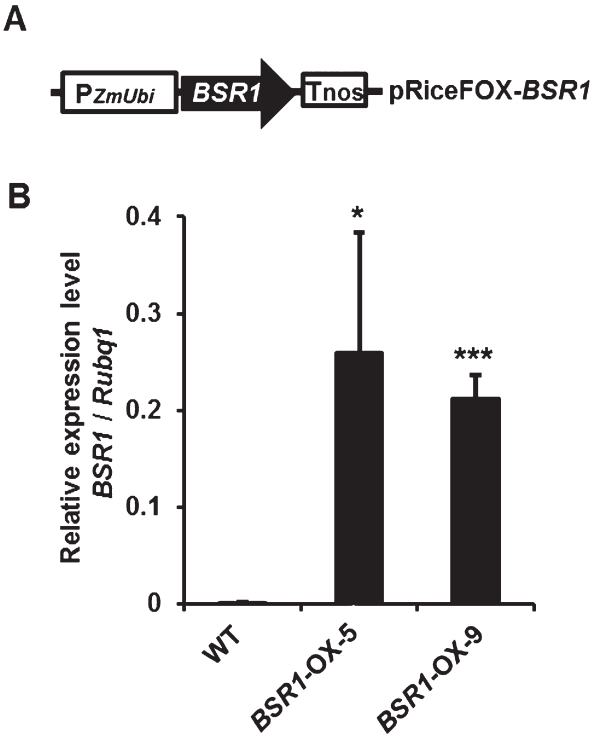


**Fig. 1.** Schematic representation of pRiceFOX-*BSR1* and the transcript level of *BSR1* in *BSR1*-OX rice. (A) The pRiceFOX-*BSR1* construct for overexpression of *BSR1*. (B) Transcript levels of *BSR1* in $T_{3–4}$ generations of *BSR1*-OX lines. Second youngest leaf blades of *BSR1*-OX and wild-type (WT) plants at the eight-leaf stage were used for measurement. Transcript levels of *BSR1* were normalized to that of an endogenous *Rubq1* housekeeping gene (Jiang *et al.* 2010). Values are means ± SD (n = 4).

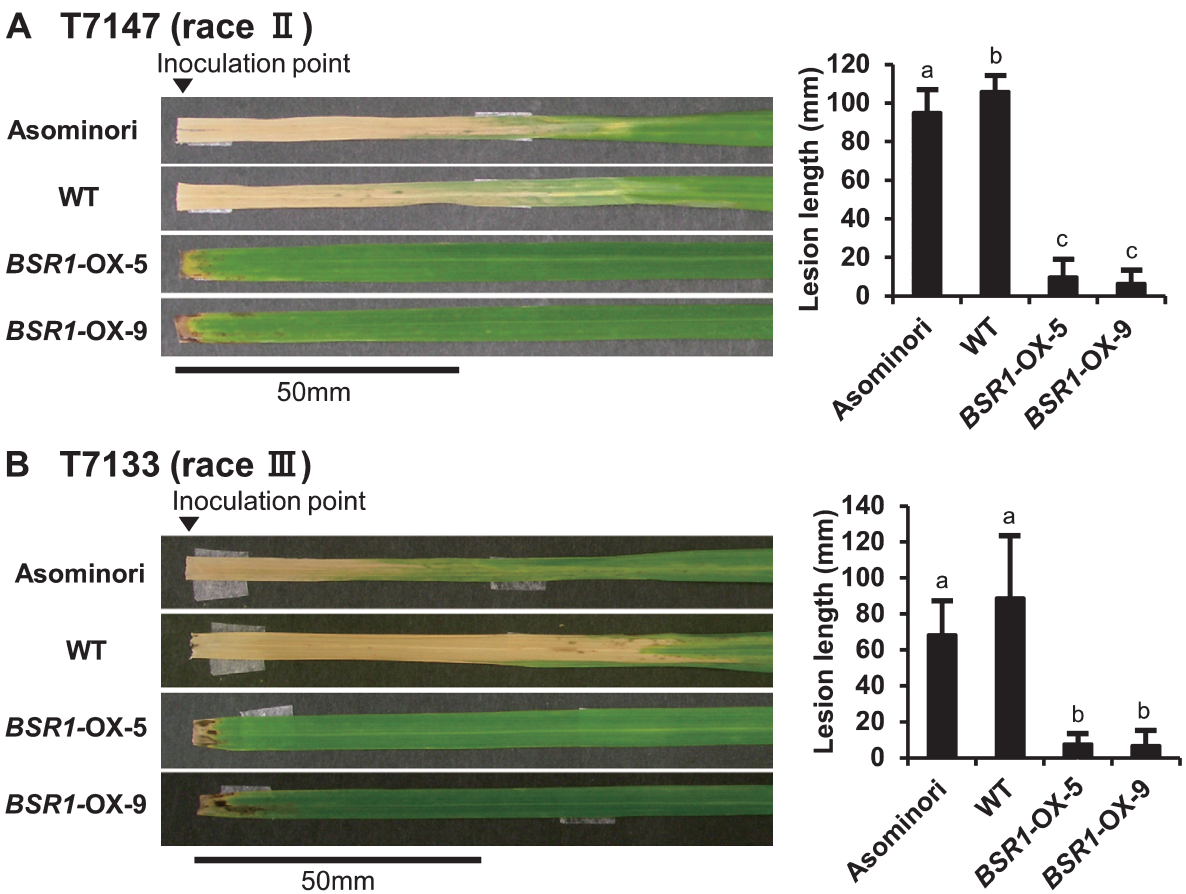


**Fig. 2.** Disease resistance to multiple races of *X. oryzae* pv. *oryzae*. Isolates T7147 (race II; A) and T7133 (race III; B) of *Xoo* were used for infection. Arrowheads indicate the point of inoculation. Lesion lengths in *BSR1*-OX plants were significantly shorter than those in wild-type (WT) and 'Asominori', the resistance control cultivar. Values are means ± SD, n = 6–18. Different letters indicate significant differences ($P < 0.05$ by Tukey-Kramer's test).

inoculated with isolate T7147 (race II) developed extended lesions from the cut (inoculated) end of the leaves, whereas *BSR1*-OX-5 and -9 plants showed restricted lesions (**Fig. 2A**). Lesion lengths in the inoculated *BSR1*-OX-5 and -9 were about 10 mm and 7 mm long, whereas those in WT and 'Asominori' were about 106 mm and 95 mm long (**Fig. 2A**). Two *BSR1*-OX lines showed 10- and 15-fold reductions in lesion length compared with the WT. Similarly, the results of inoculation of isolate T7133 (race III) are shown in **Fig. 2B**. The two *BSR1*-OX lines showed 10- and 12-fold reductions in lesion length compared with the WT. These results indicated that overexpression of *BSR1* confers strong resistance to both T7147 (race II) and T7133 (race III), as well as to previously shown T7174 (race I). The results suggested that overexpression of *BSR1* conferred non-race-specific resistance to *Xoo*. Although 'Asominori' has very strong resistance to the isolate T7174 (race I), the resistances to T7147 (race II) and T7133 (race III) were moderate (Kaku and Kimura 1989). Resistance levels of 'Asominori' shown here were similar to the report and 'Asominori' showed more race-specific resistance.

Next, to examine whether overexpression of *BSR1* also confers resistance to another race of *M. oryzae* in rice, isolate Hoku1 (MAFF101512, race 007.0) was used for inoculation. Lesion numbers in the *BSR1*-OX-5 and -9 lines were significantly smaller than those in the WT plants (**Fig. 3**). Thus, because *BSR1*-OX lines conferred strong resistance to isolate Hoku1 (race 007.0) in addition to the previously shown isolate Kyu89-246 (race 003.0), we hypothesized that overexpression of *BSR1* conferred non-race-specific resistance to *M. oryzae*.

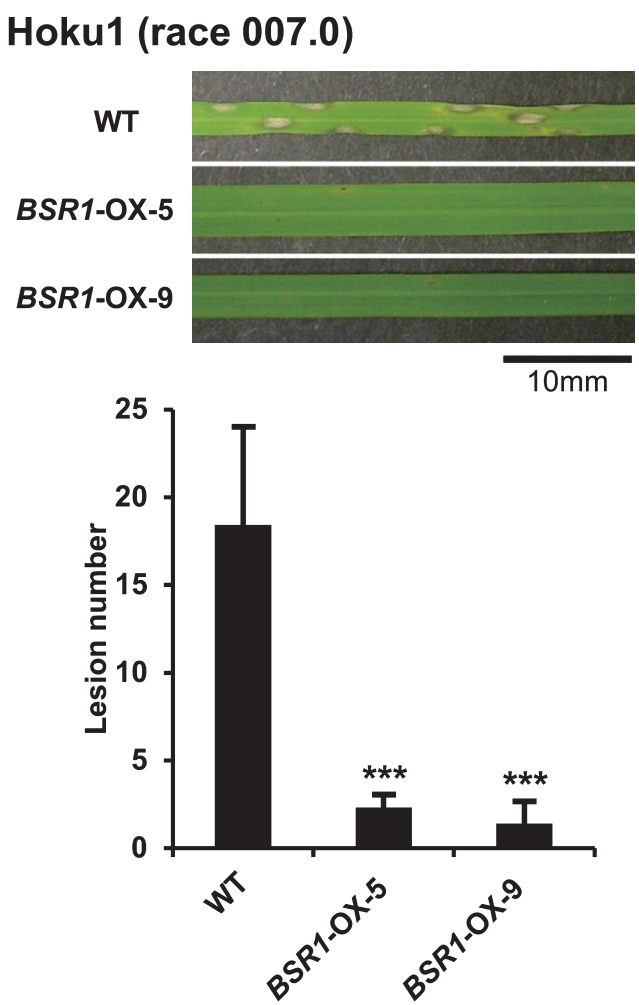


**Fig. 3.** Disease resistance to another race of *M. oryzae*. Isolate Hoku1 (race 007.0) of *M. oryzae* was used. Lesion numbers in *BSR1*-OX plants were significantly lower than those in wild-type (WT) plants (***$P < 0.001$ by t-test). Values are means ± SD, n = 3–7.

### *Extended resistance to another bacterial pathogen,* Burkholderia glumae

Bacterial seedling rot and bacterial grain rot (bacterial panicle blight) are caused by the bacterial pathogen *B. glumae*. The latter is an increasingly important disease problem in global rice production (Ham *et al.* 2011). Many genetic studies for resistance to bacterial grain rot have been reported (Mizobuchi *et al.* 2013a, 2015, Pinson *et al.* 2010, Sayler *et al.* 2006, Wasano and Okuda 1994). However, there are few reports on resistance to bacterial seedling rot, because such resistance is a complex characteristic influenced by environmental factors (Iwai *et al.* 2002, Mizobuchi *et al.* 2013b). We were interested in whether overexpression of *BSR1* conferred resistance to bacterial seedling rot in rice. In the test for resistance to bacterial seedling rot, non-germinated seeds are usually used for inoculation by soaking. However, it was difficult to evaluate the resistance by this method because *BSR1*-OX rice displayed a decreased germination rate (Dubouzet *et al.* 2011). Therefore, we gathered only pre-germinated seeds for use in the disease resistance test. To determine the condition for inoculation of pre-germinated seeds, we performed a preliminary experiment using various concentrations of *B. glumae* suspension and WT seeds. The disease symptoms were classified as shown in **Fig. 4A**. Browning of the leaf sheath was usually detected together with a dwarf phenotype in diseased plants. Disease resistance was evaluated by the survival ratio, indicating the ratio of healthy plants to total seeds used for infection and shown as a percentage. When non-germinated (NG) seeds were inoculated by soaking in suspensions of three different concentrations of *B. glumae*, no healthy plant survived in all concentrations (**Fig. 4B**). In contrast, when pre-germinated seeds were used, 98%, 43% and 2% of healthy plants survived after soaking in suspensions of low ($OD_{520} = 0.0004$), medium ($OD_{520} = 0.004$) and high ($OD_{520} = 0.04$) concentrations of the bacteria, respectively. Thus, the optimal concentration range to evaluate resistance in this experiment was $OD_{520} = 0.004$–$0.04$. In subsequent experiments, we evaluated disease resistance by this method using pre-germinated seeds.

Resistance to *B. glumae* was evaluated for *BSR1*-OX pre-germinated seeds. Survival ratios in *BSR1*-OX-5 and -9 lines were two times higher than that in the WT (**Fig. 5**).

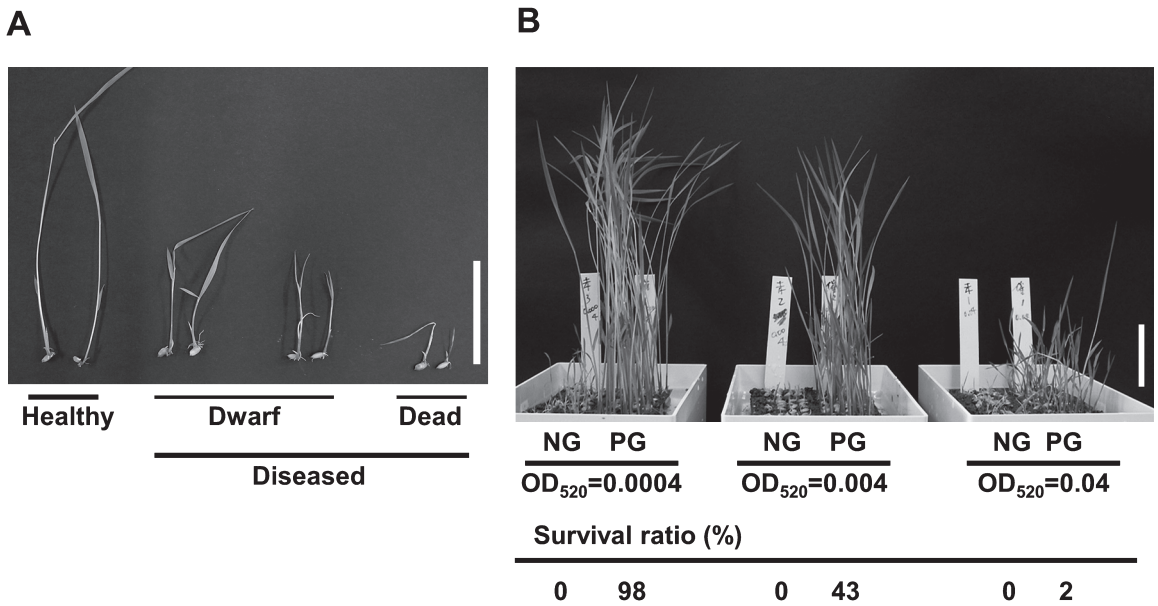


**Fig. 4.** Preliminary experiments to determine the optimal concentration of bacterial pathogen, *Burkholderia glumae*, for inoculation. (A) The classification of disease symptoms at 7 days after inoculation. Healthy, normal phenotype; Diseased, dwarf or dead phenotype. Bar = 50 mm. (B) Results of the preliminary experiment. Photograph shows plants at 7 days after inoculation (n = 50–60). Non-germinated seeds (NG) and pre-germinated seeds (PG) were used for inoculation. Bar = 50 mm. Survival ratio (%) = (number of healthy plants/number of total seeds) × 100

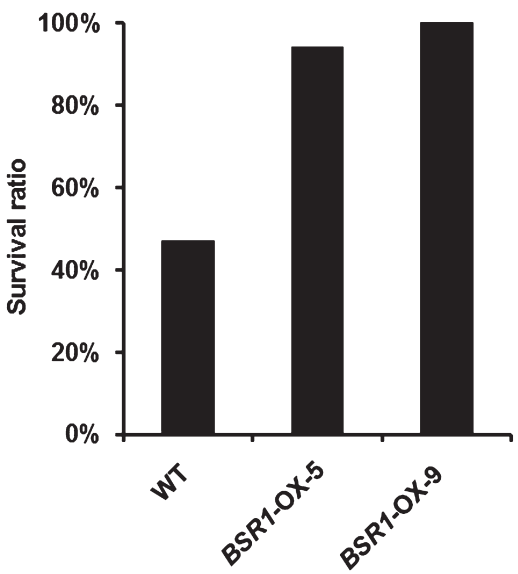


**Fig. 5.** Disease resistance to another bacterial pathogenic species, *Burkholderia glumae*. Pre-germinated seeds of *BSR1*-OX and wild-type (WT) were inoculated with *B. glumae*. Concentration of inoculation was $OD_{520}$ = 0.004. Survival ratio was calculated 8 days after inoculation (n = 17). Tests were performed three times with similar results.

These results indicated that *BSR1*-OX lines displayed resistance to *B. glumae* in rice.

### *Extended resistance to another major fungal pathogen,* Cochliobolus miyabeanus

Brown spot disease is caused by the fungal pathogen, *C. miyabeanus*. We hypothesized that *BSR1* conferred resistance to brown spot disease as well as to rice blast in rice. First, we investigated whether the expression of *BSR1* changed by inoculation with *C. miyabeanus* in WT plants (**Fig. 6A**). *C. miyabeanus* was spray-inoculated onto WT plants, and the transcript level of *BSR1* was measured by qRT-PCR. As a result, inoculated plants showed inducible expression of *BSR1* compared with mock control after inoculation (**Fig. 6A**), although the transcript levels of *BSR1* in the inoculated plants were much lower than those in *BRS1*-OX lines. This result suggested that *BSR1* is involved in innate immunity against brown spot in rice. Hence, we examined whether overexpression of *BSR1* conferred resistance to *C. miyabeanus* (**Fig. 6B**). Lesion numbers in *BSR1*-OX-5 and -9 plants were significantly lower than those in the WT plants (***$P < 0.001$ by t-test, **Fig. 6B**). Thus, overexpression of *BSR1* conferred significant resistance to *C. miyabeanus*.

### BSR1-*OX rice were slightly resistant to rice stripe virus (RSV)*

We examined whether overexpression of *BSR1* could confer resistance against a viral pathogen, RSV, in rice, because the majority of *japonica* cultivars, including cv. ‘Nipponbare’, are susceptible to RSV. The results are shown in **Fig. 7**. After inoculation of RSV, the percentages of diseased seedlings detected by ELISA in *BSR1*-OX-5 and -9 seedlings were slightly lower than those in WT seedlings, but were higher than that in cv. ‘Sainokagayaki’, the RSV-resistant control cultivar (**Fig. 7**). Thus, overexpression of *BSR1* could confer slight resistance to RSV, although its

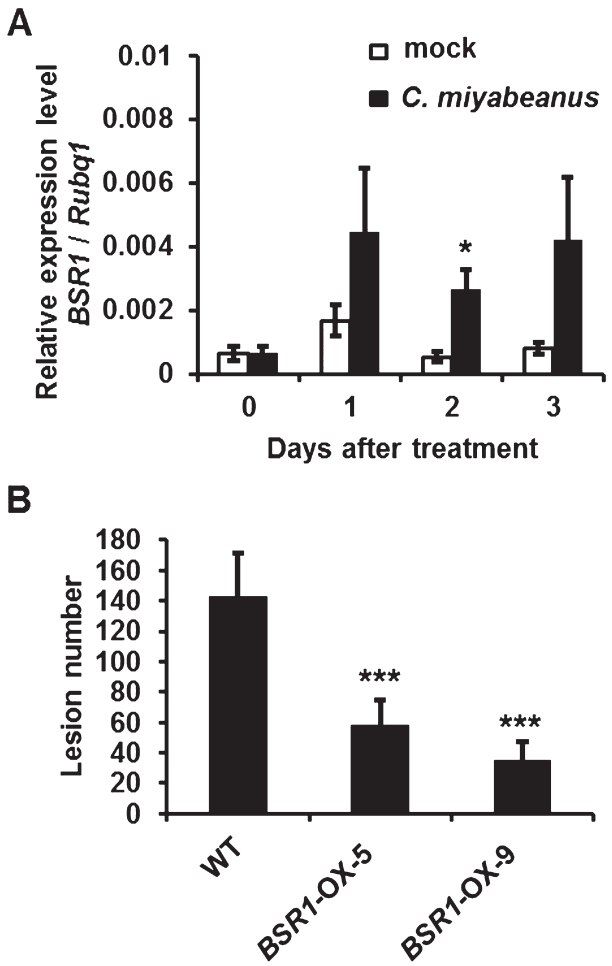


**Fig. 6.** Disease resistance to another fungal pathogenic species, *Cochliobolus miyabeanus*. (A) Relative expression levels of *BSR1* in wild-type (WT) plants on inoculation with mock or *C. miyabeanus*. Seventh-leaf blades were used for inoculation. Total RNAs at 0 to 3 days after inoculation were extracted. Transcript levels of *BSR1* were normalized to that of *Rubiq1*. Expression levels of *BSR1* in inoculated leaves were up-regulated compared with mock at 1 to 3 days. Values are means ± SD, n = 3. An asterisk indicates a statistically significant difference from the mock at 2 days ($P < 0.05$ by t-test). (B) Resistance to *C. miyabeanus* in *BSR1*-OX rice. Lesion numbers in *BSR1*-OX plants were significantly lower than those in WT plants (***$P < 0.001$ by t-test). Values are means ± SD, n = 4–12.

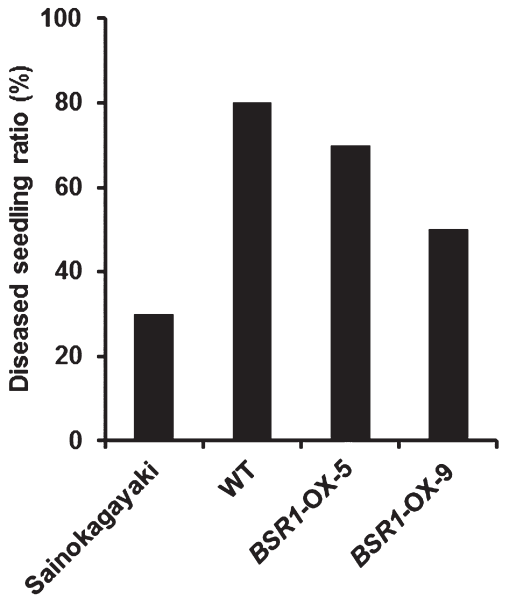


**Fig. 7.** Disease resistance to a viral pathogen, rice stripe virus. Diseased seedling ratio, percentage of diseased seedlings detected by enzyme-linked immunosorbent assay (ELISA) in all inoculated seedlings at 16 days after inoculation. n = 8 to 10. Tests were performed three times with similar results.

resistance level was weaker than that of 'Sainokagayaki', which possesses the highly resistant *Stvb-i* gene to RSV (Shimizu *et al.* 2011).

## Discussion

From the point of view of breeding, the quality of broad-spectrum resistance against two or more different pathogen species is an agronomically important trait. We previously reported that overexpression of *BSR1*, encoding a receptor-like cytoplasmic kinase, confers remarkable resistance to both bacterial and fungal pathogens in *Arabidopsis*, and that it confers resistance to the bacterial pathogen, *Xoo*, and fungal pathogen, *M. oryzae*, in rice (Dubouzet *et al.* 2011). In this study, we first showed that *BSR1* confers resistance to multiple races of *Xoo* and *M. oryzae* in overexpressing rice. In addition, *BSR1*-OX rice showed extended resistance to another bacterial pathogen, *B. glumae*, and another fungal pathogen, *C. miyabeanus*. Thus, overexpression of *BSR1* conferred broad-spectrum disease resistance to at least two bacterial and two fungal pathogenic species in rice. Therefore, it would be natural to consider that the resistance to bacterial and fungal pathogens by *BSR1* is non-race specific. Furthermore, *BSR1*-OX rice showed slight resistance to RSV. To the best of our knowledge, there are no other genes that confer such multi-disease resistance.

As a typical example of interactions with host plants and pathogens, the plant defense against pathogens, such as bacteria and fungi, is provided through cell-surface-localized pattern recognition receptors that detect pathogen-associated molecular patterns (PAMPs), and result in pattern-triggered immunity (PTI). To counteract this innate immunity, pathogens deliver effector proteins into the host cell to suppress PTI. In some cases, plants use intracellular resistance (R) proteins to detect race-specific effectors, which results in effector-triggered immunity, often associated with hypersensitive response and programmed cell death. The *BSR1* gene, *OsRLCK278*, is one of 379 RLCK genes encoding receptor-like cytoplasmic kinases in rice (Dubouzet *et al.* 2011). BSR1 is classified into the RLCK-VII protein family, which belongs to the same family as BIK1 and PBS, the well-characterized *Arabidopsis* RLCKs involved in plant defense (Dubouzet *et al.* 2011). *Arabidopsis BIK1* was originally isolated as a gene involved in the defense against

necrotrophic fungal pathogens (Veronese *et al.* 2006). BIK1 also associates with a flagellin receptor complex, FLS2/BAK1, which is rapidly phosphorylated upon perception of flagellin. In addition, BIK1 is also phosphorylated by another PAMP, translation elongation factor (EF-Tu). Hence, BIK1 is considered to mediate PTI signal transduction, such as production of a reactive oxygen species (ROS) burst, activation of mitogen-activated protein kinases (MAPKs) and calcium-dependent protein kinases (CPKs or CDPKs), transcriptional reprogramming, and ultimately immunity, from multiple PAMP receptor complexes (Chinchilla *et al.* 2007, Heese *et al.* 2007, Lu *et al.* 2010, Roux *et al.* 2011, Schulze *et al.* 2010, Schwessinger *et al.* 2011, Sun *et al.* 2013). Furthermore, BIK1 regulates calcium influx, phosphorylates and activates NADPH oxidase RBOHD, and not only induces the defense response (including the ROS burst that is a poisonous factor directly attacking pathogens), but also prevents invasion of pathogenic bacteria by closing stoma (Kadota *et al.* 2014, Li *et al.* 2014). Thus, BIK1 plays pivotal roles in recognition of PAMPs and subsequent signal transductions. In rice, OsRLCK185, which belongs to the RLCK-VII family, was reported recently (Yamaguchi *et al.* 2013). OsRLCK158 interacts with a pattern recognition receptor OsCERK1, which recognizes chitin and peptidoglycan at the plasma membrane, and regulates a MAP kinase cascade that leads to a PTI defense response. Taken together, it is likely that the BSR1 protein interacts with various transmembrane receptors that recognize PAMPs of *Xoo*, *M. oryzae*, *B. glumae* and/or *C. miyabeanus*, which functions in linking multiple PAMP receptor complexes to downstream intracellular signaling and enhances a part of PAMP-mediated defense.

From the viewpoint of the pathogen character, the lifestyles of pathogens used in this study are classified as hemibiotrophs or necrotrophs. The blast fungus, *M. oryzae*, and bacterial leaf blight bacteria pathogen, *Xoo*, are considered hemibiotrophs (Van Bockhaven *et al.* 2013). Hemibiotrophs are characterized by an initial infectious period of the biotrophic stage in which the pathogens grow within host cells before switching to a necrotrophic growth stage when lesions become apparent (Wilson and Talbot 2009). *Xoo* has been considered mostly as a biotroph but is probably best classified as a hemibiotroph (De Vleesschauwer *et al.* 2013). Furthermore, the pathogens of bacterial seedling rot, *B. glumae*, and of brown spot, *C. miyabeanus*, have been classified as necrotrophs (Iwai *et al.* 2002, Su'udi *et al.* 2012). Thus, we demonstrated that *BSR1*-OX rice conferred resistance not only to hemibiotrophs but also necrotrophs.

Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are typical defense hormones, and the regulation of their signaling networks against pathogen infection is well established, especially in the dicotyledonous model plant *Arabidopsis* (Van Bockhaven *et al.* 2013). In *Arabidopsis*, SA-dependent defenses are generally associated with resistance to biotrophs, whereas JA/ET-dependent defenses are generally associated with necrotrophs (Van Bockhaven *et al.* 2013, Thomma *et al.* 2001, De Vos *et al.* 2006). By contrast, the defense system in rice is considered to be controlled by a more complicated signaling network (De Vleesschauwer *et al.* 2013). SA-, JA- and ET-dependent defenses all enhanced resistance to hemibiotrophic *M. oryzae* (Ahn *et al.* 2005, Helliwell *et al.* 2013, Iwai *et al.* 2006, Mei *et al.* 2006, Peng *et al.* 2012, Qiu *et al.* 2007, Schweizer *et al.* 1998, Shimono *et al.* 2007, 2012). Meanwhile, SA- and JA-dependent defenses were involved in disease resistance against hemibiotrophic *Xoo* (Babu *et al.* 2003, Qiu *et al.* 2007, Shimono *et al.* 2012, Tanaka *et al.* 2014, Taniguchi *et al.* 2014), whereas ET-dependent defense played a negative role (Shen *et al.* 2011). Among the hemibiotrophs, the role of ET is the opposite of *Xoo* and *M. oryzae*. Similarly, ET-dependent defense is involved negatively in the resistance against *C. miyabeanus*, a necrotrophic pathogen (De Vleesschauwer *et al.* 2010), while no reports have implicated SA- and JA-dependent defenses against *C. miyabeanus* (Van Bockhaven *et al.* 2013). More interestingly, ABA, which antagonizes the SA pathway, is involved in the resistance to *C. miyabeanus* (De Vleesschauwer *et al.* 2010), although ABA promotes susceptibility against *Xoo* and *M. oryzae* (Jiang *et al.* 2010, Xu *et al.* 2013). The plant hormone defense network against *B. glumae* is unknown in rice. RSV resistance is implicated in enhancing the SA signal (Wang *et al.* 2014). Taken together, understanding the mechanism of broad-spectrum resistance of *BSR1* from the point of view of plant hormones is complex.

Currently, several genes have been reported to confer broad-spectrum disease resistance in rice. For example, overexpression of *OsWRKY13* or *OsWRKY45* enhanced resistance to *Xoo* and *M. oryzae* in rice by mediating SA signaling (Qiu *et al.* 2007, Shimono *et al.* 2007, 2012). Unlike *BSR1*-OX rice, however, there are few reports that overexpressed genes enhanced resistance to necrotrophic pathogens, *B. glumae* and *C. miyabeanus* in rice. Meanwhile, overexpression of *OsWRKY30* or *OsACS2* encoding a key enzyme of ET biosynthesis enhanced resistance to fungal pathogens *M. oryzae* and necrotrophic *Rhizoctonia solani* in rice. The resistance conferred by *OsWRKY30* was associated with the activation of JA synthesis-related genes and the increased accumulation of endogenous JA (Peng *et al.* 2012), and that by *OsACS2* was associated with the increased level of endogenous ET (Helliwell *et al.* 2013). Hence, we speculate that the broad-spectrum disease resistance against two hemibiotrophs (*Xoo* and *M. oryzae*) and two necrotrophs (*B. glumae* and *C. miyabeanus*) in *BSR1*-OX rice is based on an SA and JA/ET combined pathway.

Here, *BSR1*-OX rice displayed strong resistance to three tested races (races I to III) of *Xoo*. In contrast, 'Asominori' showed more race-specific resistance. Although the resistance to isolate T7174 (race I) on 'Asominori' was very strong (Dubouzet *et al.* 2011), the resistances to T7147 (race II) and T7133 (race III) were moderate (**Fig. 2**), almost similar to the report of Kaku and Kimura (1989). 'Asominori' has been reported to have an *Xa1-as*(t) gene at the *Xa1* locus

(Ise *et al.* 1998). *Xa1-as*(t) is implicated in the strong resistance to T7174 (race I). 'Asominori' has also been suggested to have minor-affected loci that are involved in the quantitative resistance to T7133 (race III) (Yoshimura *et al.* 1996). *BSR1-OX* rice showed strong resistance to both T7174 (race I) and T7133 (race III); therefore, the defense mechanism of *BSR1*-OX rice would be different from that of 'Asominori'. From the viewpoint of breeding, overexpression of *BSR1* could confer more useful non-race-specific resistance to *Xoo* in rice compared with using the resistance genes of 'Asominori'.

In the previous report, *BSR1*-OX rice displayed strong resistance to race 003.0 (isolate Kyu89-246) and the resistance level was stronger than that in cv. 'Sensho' (Dubouzet *et al.* 2011), which has a strong non-race-specific resistance to *M. oryzae* associated with *pi21* (Fukuoka *et al.* 2009). In this paper, *BSR1*-OX rice displayed extended strong resistance to race 007.0 (isolate Hoku1; **Fig. 3**). Hence, non-race-specific resistance or field resistance against *M. oryzae* is also promising in *BSR1*-OX rice. Taken together, overexpression of *BSR1* could confer more promising leaf blight and blast resistances compared with the resistant cultivars 'Asominori' and 'Sensho', respectively, in many useful *O. sativa* varieties. Furthermore, it is plausible that *BSR1*-OX rice also shows non-race-specific resistance to *B. glumae* and *C. miyabeanus*, and exhibits resistance to other pathogen species, because *BSR1*-OX rice showed resistance to all pathogens tested.

In conclusion, *BSR1*, when overexpressed in rice, conferred broad-spectrum disease resistance against at least four diseases: bacterial leaf blight, blast, bacterial seedling rot and brown spot, and slight resistance against rice stripe disease by RSV. *BSR1* represents a highly valuable and convenient genetic resource because it confers resistance to various diseases by a single gene. In the future, the defense mechanism conferred by *BSR1* will be clarified to use the *BSR1* gene effectively.

## Acknowledgements

This work was supported by a grant from the Ministry of Agriculture, Forestry, and Fisheries of Japan (Genomics-based Technology for Agricultural Improvement, GMO-1006). We thank Dr. Hiroaki Ichikawa (NIAS, Japan) for providing the pRiceFOX vector, Dr. Hirokazu Ochiai (NIAS, Japan) for providing *Xoo* and 'Asominori' rice seeds, Dr. Koji Azegami (NARC, Japan) for providing *B. glumae* and Dr. Ritsuko Mizobuchi (Institute of Crop Science, NARO, Japan) for advice on the *B. glumae* assay. We also thank Lois Ishizaki, Tomiko Senba and Chiyoko Umeda (NIAS, Japan) for their support during the rice transformation experiments and for overall technical assistance.

## Literature Cited

Ahn, I.P., S. Kim, S. Kang, S.C. Suh and Y.H. Lee (2005) Rice defense mechanisms against *Cochliobolus miyabeanus* and *Magnaporthe grisea* are distinct. Phytopathology 95: 1248–1255.

Babu, R.M., A. Sajeena, A.V. Samundeeswari, A. Sreedhar, P. Vidhyasekaran, K. Seetharaman and M.S. Reddy (2003) Induction of systemic resistance to *Xanthomonas oryzae* pv. *oryzae* by salicylic acid in *Oryza sativa* (L.). J. Plant Dis. Prot. 110: 419–431.

Chinchilla, D., C. Zipfel, S. Robatzek, B. Kemmerling, T. Nürnberger, J.D. Jones, G. Felix and T. Boller (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature 448: 497–500.

De Vleesschauwer, D., Y. Yang, C.V. Cruz and M. Höfte (2010) Abscisic acid-induced resistance against the brown spot pathogen *Cochliobolus miyabeanus* in rice involves MAP kinase-mediated repression of ethylene signaling. Plant Physiol. 152: 2036–2052.

De Vleesschauwer, D., G. Gheysen and M. Höfte (2013) Hormone defense networking in rice: tales from a different world. Trends Plant Sci. 18: 555–565.

De Vos, M., W. Van Zaanen, A. Koornneef, J.P. Korzelius, M. Dicke, L.C. Van Loon and C.M. Pieterse (2006) Herbivore-induced resistance against microbial pathogens in Arabidopsis. Plant Physiol. 142: 352–363.

Dean, R.A., N.J. Talbot, D.J. Ebbole, M.L. Farman, T.K. Mitchell, M.J. Orbach, M. Thon, R. Kulkarni, J.R. Xu, H. Pan *et al.* (2005) The genome sequence of the rice blast fungus *Magnaporthe grisea*. Nature 434: 980–986.

Dubouzet, J.G., S. Maeda, S. Sugano, M. Ohtake, N. Hayashi, T. Ichikawa, Y. Kondou, H. Kuroda, Y. Horii, M. Matsui *et al.* (2011) Screening for resistance against *Pseudomonas syringae* in rice-FOX *Arabidopsis* lines identified a putative receptor-like cytoplasmic kinase gene that confers resistance to major bacterial and fungal pathogens in *Arabidopsis* and rice. Plant Biotechnol. J. 9: 466–485.

Flor, H.H. (1971) Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. 9: 275–296.

Fukuoka, S., N. Saka, H. Koga, K. Ono, T. Shimizu, K. Ebana, N. Hayashi, A. Takahashi, H. Hirochika, K. Okuno *et al.* (2009) Loss of function of a proline-containing protein confers durable disease resistance in rice. Science 325: 998–1001.

Ham, J.H., R.A. Melanson and M.C. Rush (2011) *Burkholderia glumae*: next major pathogen of rice? Mol. Plant Pathol. 12: 329–339.

Heese, A., D.R. Hann, S. Gimenez-Ibanez, A.M. Jones, K. He, J. Li, J.I. Schroeder, S.C. Peck and J.P. Rathjen (2007) The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. Proc. Natl. Acad. Sci. USA 104: 12217–12222.

Helliwell, E.E., Q. Wang and Y. Yang (2013) Transgenic rice with inducible ethylene production exhibits broad-spectrum disease resistance to the fungal pathogens *Magnaporthe oryzae* and *Rhizoctonia solani*. Plant Biotechnol. J. 11: 33–42.

Hsing, Y.I., C.G. Chern, M.J. Fan, P.C. Lu, K.T. Chen, S.F. Lo, P.K. Sun, S.L. Ho, K.W. Lee, Y.C. Wang *et al.* (2007) A rice gene activation/knockout mutant resource for high throughput functional genomics. Plant Mol. Biol. 63: 351–364.

Ise, K., C.Y. Li, Y.Q. Sun and C.R. Ye (1998) Inheritance of resistance to bacterial leaf blight in differential rice variety Asominori. IRRN 23: 13–14.

Iwai, T., H. Kaku, R. Honkura, S. Nakamura, H. Ochiai, T. Sasaki and Y. Ohashi (2002) Enhanced resistance to seed-transmitted bacterial diseases in transgenic rice plants overproducing an oat cell-wall-bound thionin. Mol. Plant Microbe Interact. 15: 515–521.

Iwai, T., A. Miyasaka, S. Seo and Y. Ohashi (2006) Contribution of ethylene biosynthesis for resistance to blast fungus infection in young

rice plants. Plant Physiol. 142: 1202–1215.

Jeong, D.H., S. An, H.G. Kang, S. Moon, J.J. Han, S. Park, H.S. Lee, K. An and G. An (2002) T-DNA insertional mutagenesis for activation tagging in rice. Plant Physiol. 130: 1636–1644.

Jiang, C.J., M. Shimono, S. Sugano, M. Kojima, K. Yazawa, R. Yoshida, H. Inoue, N. Hayashi, H. Sakakibara and H. Takatsuji (2010) Abscisic acid interacts antagonistically with salicylic acid signaling pathway in rice-*Magnaporthe grisea* interaction. Mol. Plant Microbe Interact. 23: 791–798.

Kadota, Y., J. Sklenar, P. Derbyshire, L. Stransfeld, S. Asai, V. Ntoukakis, J.D. Jones, K. Shirasu, F. Menke, A. Jones *et al.* (2014) Direct regulation of the NADPH oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. Mol. Cell 54: 43–55.

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

Kondou, Y., M. Higuchi, S. Takahashi, T. Sakurai, T. Ichikawa, H. Kuroda, T. Yoshizumi, Y. Tsumoto, Y. Horii, M. Kawashima *et al.* (2009) Systematic approaches to using the FOX hunting system to identify useful rice genes. Plant J. 57: 883–894.

Li, L., M. Li, L. Yu, Z. Zhou, X. Liang, Z. Liu, G. Cai, L. Gao, X. Zhang, Y. Wang *et al.* (2014) The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RbohD to control plant immunity. Cell Host Microbe 15: 329–338.

Liu, W., J. Liu, L. Triplett, J.E. Leach and G.L. Wang (2014) Novel insights into rice innate immunity against bacterial and fungal pathogens. Annu. Rev. Phytopathol. 52: 213–241.

Lu, D., S. Wu, X. Gao, Y. Zhang, L. Shan and P. He (2010) A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. Proc. Natl. Acad. Sci. USA 107: 496–501.

Mei, C., M. Qi, G. Sheng and Y. Yang (2006) Inducible overexpression of a rice allene oxide synthase gene increases the endogenous jasmonic acid level, *PR* gene expression, and host resistance to fungal infection. Mol. Plant Microbe Interact. 19: 1127–1137.

Mizobuchi, R., H. Sato, S. Fukuoka, T. Tanabata, S. Tsushima, T. Imbe and M. Yano (2013a) Mapping a quantitative trait locus for resistance to bacterial grain rot in rice. Rice (N Y) 6: 13.

Mizobuchi, R., H. Sato, S. Fukuoka, S. Tsushima, T. Imbe and M. Yano (2013b) Identification of *qRBS1*, a QTL involved in resistance to bacterial seedling rot in rice. Theor. Appl. Genet. 126: 2417–2425.

Mizobuchi, R., H. Sato, S. Fukuoka, S. Tsushima and M. Yano (2015) Fine mapping of *RBG2*, a quantitative trait locus for resistance to *Burkholderia glumae*, on rice chromosome 1. Mol. Breed. 35: 15.

Moletti, M., M.L. Giudici and B. Villa (1997) Rice Akiochi-brown spot disease in Italy: Agronomic and chemical control. *In*: Chataigner, J. (ed.) Maladies du riz en région méditerranéenne et les possibilités d' amélioration de sa résistance, CIHEAM, Montpellier, pp. 79–85.

Mori, M., C. Tomita, K. Sugimoto, M. Hasegawa, N. Hayashi, J.G. Dubouzet, H. Ochiai, H. Sekimoto, H. Hirochika and S. Kikuchi (2007) Isolation and molecular characterization of a *Spotted leaf 18* mutant by modified activation-tagging in rice. Plant Mol. Biol. 63: 847–860.

Nakamura, H., M. Hakata, K. Amano, A. Miyao, N. Toki, M. Kajikawa, J. Pang, N. Higashi, S. Ando, S. Toki *et al.* (2007) A genome-wide gain-of function analysis of rice genes using the FOX-hunting system. Plant Mol. Biol. 65: 357–371.

Noda, S., T. Omura, M. Murakami and T. Tsuchizaki (1991) Infectivity of rice viruses to the varieties resistant to rice stripe virus. Ann. Phytopathol. Soc. Jpn. 57: 259–262.

Peng, X., Y. Hu, X. Tang, P. Zhou, X. Deng, H. Wang and Z. Guo (2012) Constitutive expression of rice *WRKY30* gene increases the endogenous jasmonic acid accumulation, *PR* gene expression and resistance to fungal pathogens in rice. Planta 236: 1485–1498.

Pinson, S.R., A.K. Shahjahan, M.C. Rush and D.E. Groth (2010) Bacterial panicle blight resistance QTLs in rice and their association with other disease resistance loci and heading date. Crop Sci. 50: 1287–1297.

Qiu, D., J. Xiao, X. Ding, M. Xiong, M. Cai, Y. Cao, X. Li, C. Xu and S. Wang (2007) OsWRKY13 mediates rice disease resistance by regulating defense-related genes in salicylate- and jasmonate-dependent signaling. Mol. Plant Microbe Interact. 20: 492–499.

Roux, M., B. Schwessinger, C. Albrecht, D. Chinchilla, A. Jones, N. Holton, F.G. Malinovsky, M. Tör, S. de Vries and C. Zipfel (2011) The *Arabidopsis* leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. Plant Cell 23: 2440–2455.

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.

Satoh, K., H. Kondoh, T. Sasaya, T. Shimizu, I.R. Choi, T. Omura and S. Kikuchi (2010) Selective modification of rice (*Oryza sativa*) gene expression by rice stripe virus infection. J. Gen. Virol. 91: 294–305.

Sayler, R.J., R.D. Cartwright and Y. Yang (2006) Genetic characterization and real-time PCR detection of *Burkholderia glumae*, a newly emerging bacterial pathogen of rice in the United States. Plant Dis. 90: 603–610.

Schulze, B., T. Mentzel, A.K. Jehle, K. Mueller, S. Beeler, T. Boller, G. Felix and D. Chinchilla (2010) Rapid heteromerization and phosphorylation of ligand-activated plant transmembrane receptors and their associated kinase BAK1. J. Biol. Chem. 285: 9444–9451.

Schweizer, P., A. Buchala, R. Dudler and J.P. Métraux (1998) Induced systemic resistance in wounded rice plants. Plant J. 14: 475–481.

Schwessinger, B., M. Roux, Y. Kadota, V. Ntoukakis, J. Sklenar, A. Jones and C. Zipfel (2011) Phosphorylation-dependent differential regulation of plant growth, cell death, and innate immunity by the regulatory receptor-like kinase BAK1. PLoS Genet. 7: e1002046.

Shen, X., H. Liu, B. Yuan, X. Li, C. Xu and S. Wang (2011) OsEDR1 negatively regulates rice bacterial resistance via activation of ethylene biosynthesis. Plant Cell Environ. 34: 179–191.

Shimizu, T., E. Nakazono-Nagaoka, T. Uehara-Ichiki, T. Sasaya and T. Omura (2011) Targeting specific genes for RNA interference is crucial to the development of strong resistance to rice stripe virus. Plant Biotechnol. J. 9: 503–512.

Shimono, M., S. Sugano, A. Nakayama, C.J. Jiang, K. Ono, S. Toki and H. Takatsuji (2007) Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance. Plant Cell 19: 2064–2076.

Shimono, M., H. Koga, A. Akagi, N. Hayashi, S. Goto, M. Sawada, T. Kurihara, A. Matsushita, S. Sugano, C.J. Jiang *et al.* (2012) Rice WRKY45 plays important roles in fungal and bacterial disease resistance. Mol. Plant Pathol. 13: 83–94.

Sun, Y., L. Li, A.P. Macho, Z. Han, Z. Hu, C. Zipfel, J.M. Zhou and J. Chai (2013) Structural basis for flg22-induced activation of the *Arabidopsis* FLS2-BAK1 immune complex. Science 342: 624–628.

Su'udi, M., J.M. Park, W.R. Kang, S.R. Park, D.J. Hwang and I.P. Ahn (2012) Quantification of rice brown leaf spot through Taqman real-time PCR specific to the unigene encoding *Cochliobolus miyabeanus* SCYTALONE DEHYDRATASE1 involved in fungal

melanin biosynthesis. J. Microbiol. 50: 947–954.

Tanaka, K., S. Taniguchi, D. Tamaoki, K. Yoshitomi, K. Akimitsu and K. Gomi (2014) Multiple roles of plant volatiles in jasmonate-induced defense response in rice. Plant Signal. Behav. 9: e29247.

Taniguchi, S., Y. Hosokawa-Shinonaga, D. Tamaoki, S. Yamada, K. Akimitsu and K. Gomi (2014) Jasmonate induction of the monoterpene linalool confers resistance to rice bacterial blight and its biosynthesis is regulated by JAZ protein in rice. Plant Cell Environ. 37: 451–461.

Thomma, B.P., I.A. Penninckx, W.F. Broekaert and B.P. Cammue (2001) The complexity of disease signaling in *Arabidopsis*. Curr. Opin. Immunol. 13: 63–68.

Van Bockhaven, J., D. De Vleesschauwer and M. Höfte (2013) Towards establishing broad-spectrum disease resistance in plants: silicon leads the way. J. Exp. Bot. 64: 1281–1293.

Veronese, P., H. Nakagami, B. Bluhm, S. Abuqamar, X. Chen, J. Salmeron, R.A. Dietrich, H. Hirt and T. Mengiste (2006) The membrane-anchored *BOTRYTIS-INDUCED KINASE1* plays distinct roles in *Arabidopsis* resistance to necrotrophic and biotrophic pathogens. Plant Cell 18: 257–273.

Wang, Q., Y. Liu, J. He, X. Zheng, J. Hu, Y. Liu, H. Dai, Y. Zhang, B. Wang, W. Wu *et al.* (2014) *STV11* encodes a sulphotransferase and confers durable resistance to rice stripe virus. Nat. Commun. 5: 4768.

Wasano, K. and S. Okuda (1994) Evaluation of resistance of rice cultivars to bacterial grain rot by the syringe inoculation method. Breed. Sci. 44: 1–6.

Wilson, R.A. and N.J. Talbot (2009) Under pressure: investigating the biology of plant infection by *Magnaporthe oryzae*. Nat. Rev. Microbiol. 7: 185–195.

Xu, J., K. Audenaert, M. Hofte and D. De Vleesschauwer (2013) Abscisic acid promotes susceptibility to the rice leaf blight pathogen *Xanthomonas oryzae* pv *oryzae* by suppressing salicylic acid-mediated defenses. PLoS ONE 8: e67413.

Yamaguchi, K., K. Yamada, K. Ishikawa, S. Yoshimura, N. Hayashi, K. Uchihashi, N. Ishihama, M. Kishi-Kaboshi, A. Takahashi, S. Tsuge *et al.* (2013) A receptor-like cytoplasmic kinase targeted by a plant pathogen effector is directly phosphorylated by the chitin receptor and mediates rice immunity. Cell Host Microbe 13: 347–357.

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

Zhang, Y.X., Q. Wang, L. Jiang, L.L. Liu, B.X. Wang, Y.Y. Shen, X.N. Cheng and J.M. Wan (2011) Fine mapping of $qSTV11^{KAS}$, a major QTL for rice stripe disease resistance. Theor. Appl. Genet. 122: 1591–1604.