DNA markers linked to Pga1, an adzuki bean gene that confers resistance to Cadophora gregata race 1

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Brown stem rot (BSR) caused by Cadophora gregata f. sp. adzukicola (syn. Phialophora gregata) is a serious soilborne disease of adzuki bean (Vigna angularis) in Japan. Cultivation of resistant cultivars is the most effective disease control method, therefore the selection of resistant lines is a priority for breeders. BSR-resistant adzuki bean lines have been screened in pathogen-infected fields. However, field selection using the pathogen and artificial inoculation methods is time-consuming and labor-intensive. In the present study, we used 105 F3 lines derived from a cross between a BSR-resistant cultivar ‘Syumari’ and a susceptible cultivar ‘Buchishoryukei-1’ for BSR inoculation tests. Amplified fragment-length polymorphism (AFLP) analyses with 1024 primer sets revealed that six fragments were polymorphic between resistance and susceptible bulked groups. Five DNA markers (Pg77, Pg118, Pg138, Pg139 and Pg126) were developed from the nucleotide sequences of polymorphic AFLP markers and their flanking regions. Pg118, which was derived from E-ACT/M-ACT-118, was tightly linked to the resistance gene Pga1 and was converted into a codominant marker for its easier use in marker-assisted selection for adzuki bean BSR resistance. Finally, the applicability of the developed markers for BSR resistance was tested on 32 adzuki bean accessions or cultivars.

Key Words: adzuki bean, brown stem rot, DNA marker, Cadophora gregata, resistance gene.

Introduction

Brown stem rot (BSR) of adzuki bean [Vigna angularis (Willd.) Ohwi & Ohashi] caused by the soilborne fungus Cadophora gregata Harrington & McNew (Harrington and McNew 2003) [syn. Phialophora gregata (Allington & D.W. Chamberlain) W. Gams] f. sp. adzukicola Kobayashi, Yamamoto, Negishi and Ogoshi (Kobayashi et al. 1991), is a serious disease in Hokkaido, Japan’s northern island (Kondo et al. 1998, 2004). Symptoms of BSR comprise wilting and a reddish-brown discoloration of the vascular and pith tissues of the stem and petioles, which often occur in conjunction with leaf chlorosis, necrosis at seed maturity, or both. Cadophora gregata isolates were obtained from commercial fields on Hokkaido from 1997 to 1999. Of these, 78.1% were rated as race 1, 13.9% as race 2 and 8.0% as race 3 (Fujita 2007, Kondo et al. 2005). BSR causes yield reductions and reduces crop quality because the infected plants produce fewer seed pods and reduced grain weight (Fujita 2007, Kondo et al. 2004). Cultivation of cultivars resistant to this disease is the most effective control method because chemical controls are ineffective, expensive, or both (Fujita 2007). Therefore it is important to develop BSR-resistant cultivars in adzuki bean breeding programs.

Research on BSR-resistant cultivars and the development of resistant cultivars have been undertaken since 1976 at the Tokachi Agricultural Experiment Station, where an adzuki bean germplasm collection which include foreign accessions and wild relatives, is utilized for breeding (Fujita et al. 2007, Kondo et al. 2004, Kondo and Tomooka 2012). Chiba (1982) previously identified highly BSR-resistant cultivars such as ‘Kuroshozu’ (Okayama), ‘Maruba’ (Kari 63) and ‘Shochohin-10’ by means of field evaluations. Furthermore, Chiba et al. (1987) reported the resistance from ‘Kuroshozu’ was inherited as a single dominant gene. Takeda et al. (2006) reported that a gene for BSR resistance carried by ‘Syumari’; the resistance gene derived from ‘Kuroshozu’ (Okayama) was designated Pga1. Screening of BSR-
resistant adzuki bean lines has been carried out in pathogen-infected fields. However, field selection with the pathogen and artificial inoculation methods are time-consuming and labor-intensive.

For effective identification of resistant plants without requiring inoculation and monitoring to detect symptoms, DNA markers can be used in marker-assisted selection (MAS) programs to aid with the introduction of BSR resistance into adzuki bean lines and cultivars. DNA markers linked to a trait of interest in plants can be identified rapidly by means of bulked segregant analysis (Michelmore et al. 1991). First, two bulks with contrasting characteristics are developed: within each bulk, the individuals are identical for the trait or gene of interest but other genetic backgrounds are not controlled and therefore likely to be heterogeneous. The two bulks are then analyzed for DNA markers to identify the markers that distinguish them. Furthermore, amplified fragment-length polymorphism (AFLP) markers have been widely used for identification of markers associated with traits of interest because this method allows a high number of restriction fragments to be visualized by means of the polymerase chain reaction (PCR) without prior knowledge of their nucleotide sequences (Vos et al. 1995).

In the present study, we had the following objectives: (1) to identify AFLP markers located near the BSR resistance gene *Pga1*; (2) to develop DNA markers for BSR resistance and evaluate the utility of these markers and (3) to convert the closest marker into a codominant marker that could be used in breeding programs.

**Material and Methods**

**Plant materials**

We used 105 F1 lines derived from a cross between *V. angularis* cv. ‘Symari’ and cv. ‘Buchishoryukei-1’ to develop DNA markers. ‘Symari’, which is derived from cv. ‘Kuroshozu’ (Okayama), is resistant to race 1 of *C. gregata* f. sp. *adzukicola* (Fujita et al. 2002), whereas ‘Buchishoryukei-1’ is highly susceptible to BSR (Fujita 2007). In addition, we used 4278 F2 plants and their progeny derived from the same cross combination between ‘Symari’ and ‘Buchishoryukei-1’ to find the most closely linked marker. Finally, we used the 35 adzuki bean accessions and cultivars listed in Table 1 to confirm the general applicability of the developed markers.

**Evaluation of BSR resistance**

We used *Cadophora gregata* f. sp. *adzukicola* isolate T96-1 (race 1; NIAS Genebank accession no. MAFF 241056) for the inoculation tests. Inoculum was produced in liquid V8 juice medium (Kondo et al. 2009) incubated at 25°C on a reciprocal shaker at 120 oscillations/min. After 3 weeks, mycelia and spores were collected by filtration through filter paper (Whatman No. 1) and then washed by suspension in distilled water, followed by filtration. The fungal pellets were resuspended in distilled water and homogenized at 10,000 rpm for 3 min with a Polytron (Kinematica, Lucerne, Switzerland) and then diluted to 10^7 spores and mycelial fragments per milliliter of distilled water according to the method of Kondo et al. (2009).

We grew five seedlings in each of three unglazed pottery pots (12.4-cm diameter) containing Takii seedling soil (Takii, Kyoto, Japan) per line for 10 to 14 days until development of the primary leaf in a greenhouse. We then washed the roots gently with running tap water and removed a half of hair roots with scissors before inoculation with the pathogen. We dipped the roots of 12 vigorous seedlings from each line into the inoculum suspension (50 mL) for 16 hours and then placed the seedlings into soil in unglazed pottery pots (12.4-cm diameter, four seedlings per pot). We then poured the remaining inoculum over the roots of the seedlings to maximize coverage evenly. Disease evaluation was assessed about 7 weeks after inoculation by observation of longitudinal section of the stem cutting by a blade; individual plants that showed browning in the vascular bundle were classified as susceptible and those without browning were classified as resistant. For construction of the bulks used in the genetic analysis, we selected F1 lines in which all plants showed either resistance or susceptibility.

**AFLP analysis**

DNA was extracted by the CTAB method (Murray and Thompson 1980) with modifications. Two bulked DNA samples, one for the resistance and the other for the susceptibility, were developed from 12 resistant or susceptible F1 plants. Each F1 plant was selected from one F3 line. AFLP analysis was carried out according to the method of Vos et al. (1995), with slight modification. Of the 124 AFLP primers sets used in this study, the EcoRI side primers were labeled with the fluorescent dyes FAM or VIC (Applied Biosystems, Foster City, CA, USA). Selective amplification was performed using various combinations of EcoRI (E) primers with three selective nucleotides and MseI primers with three selective nucleotides (E-NNN/M-NNN). The amplification products were separated using an ABI Prism 310 Genetic Analyzer (Applied Biosystems) with the GeneScan software and the GeneScan-300 LIZ size standard. We selected AFLP primer sets that produced fragments specific to either of the resistant or susceptible bulks.

**Development of DNA markers**

Amplified fragments that showed polymorphisms between the bulks in the AFLP analysis were extracted from polyacrylamide gels (8%) and crushed in a diffusion buffer (0.5 M ammonium acetate, 10 mM magnesium acetate, 1 mM EDTA [pH 8.0] and 0.1% SDS) with a glass stick. Amplified fragments were purified with a Superrec-01 spin column (Takara, Shiga, Japan) and concentrated using the MiniElute PCR Purification Kit (Qiagen, Hilden, Germany) for sequencing. The flanking region of the AFLP fragment was sequenced by means of inverse PCR (Ochman et al. 1988). Inverse PCR was carried out for the DNA samples
DNA markers linked to adzuki BSR resistance gene

We could not amplify the region of Pg138-'Buchishoryukei-1'. The amplified regions sequenced in this study were submitted to the DDBJ (Accession no. from AB749324 to AB749332). Sequence characterized amplified region (SCAR) markers were developed to amplify the DNA fragment only from the resistant plants. In this study the melting temperature and secondary structure of all PCR primers were checked by using the DNA Calculator tool (http://www.sigma-genosys.com/calc/DNACalc.asp). The length of primers was 18 to 28 nucleotides, the GC content was 35 to 40% and the optimum melting temperature was 60 ± 5°C in order for annealing in PCR reactions to be performed at 55°C or 60°C. The PCR solution contained 0.2 mM each dNTP, 1.25 U Takara Taq, 1× reaction buffer, 0.4 μM each primer and 50 ng template DNA in a total volume of 50 μl. The PCR protocol was as follows: 1 cycle of 95°C for 2 min; 35 cycles of 95°C for 30 s, 60°C or 55°C (in case of melting temperature was below 60°C and the sample did not amplify in 60°C) for 30 s, and 72°C for 2 min and 1 cycle of 72°C for 5 min.

**Selection of the marker closest to Pg118**

We genotyped 4278 F2 plants derived from 30 F1 plants of the crosses between ‘Syumari’ and ‘Buchishoryukei-1’ using the developed markers. The F2 plants that showed recombination between the markers were harvested. Six plants of the F3 progeny from the ten F2 plants selected were genotyped by the SCAR markers, and their BSR-resistance phenotype was evaluated using the inoculation test.

### Table 1. BSR resistances and SCAR marker genotypes of adzuki bean accessions and cultivars in Japan

| Name                  | Accession or cultivar | BSR resistance | SCAR marker genotype | Source of the resistance |
|-----------------------|-----------------------|----------------|----------------------|--------------------------|
|                       |                       |                | Pg118b | Pg139b | Pg118c | Pg126c | Pg77c | Pg138c |
| Buchishoryukei-1      | accession             | S              | B      | -      | -      | -      | -      | -      |
| Kensaki local variety | S                     | S              | B      | +      | -      | -      | -      | -      |
| Chagarawase local variety | S              | S              | B      | +      | -      | -      | -      | -      |
| Wase-maruba local variety | S              | S              | B      | -      | -      | -      | -      | -      |
| Wasedairyu local variety | S               | S              | B      | -      | -      | -      | -      | -      |
| Takahashi-wase cultivar | S                  | B              | -      | -      | -      | -      | -      | -      |
| Kuroshozu (Okayama) accession | R          | R              | S      | +      | +      | +      | +      | +      |
| Maruba (Kari 63) accession | R               | R              | S      | +      | +      | +      | +      | +      |
| Shochohin-10 accession | R                   | R              | S      | -      | +      | +      | +      | +      |
| Akamine accession     | R                     | R              | S      | -      | -      | -      | -      | -      |
| Acc259 accession      | R                     | R              | S      | -      | -      | -      | -      | -      |
| Takarashozu cultivar  | S                     | S              | B      | +      | -      | -      | -      | -      |
| Hikarishozu cultivar  | S                     | S              | B      | +      | -      | -      | -      | -      |
| Kotobukishozu cultivar | S                   | S              | B      | -      | -      | -      | -      | -      |
| Sakaeshozu cultivar   | S                     | S              | B      | +      | -      | -      | -      | -      |
| Hayateshohzu cultivar | S                     | S              | B      | +      | -      | -      | -      | -      |
| Erimoshozu cultivar   | S                     | S              | B      | -      | -      | -      | -      | -      |
| Hatunemeshozu cultivar | S                  | S              | B      | -      | -      | -      | -      | -      |
| Sahoroshozu cultivar  | S                     | S              | B      | +      | -      | -      | -      | -      |
| Akeno-wase cultivar   | S                     | S              | B      | -      | -      | -      | -      | -      |
| Kitano-otome cultivar | R                     | R              | S      | +      | +      | +      | +      | +      |
| Syumari cultivar      | R                     | R              | S      | +      | +      | +      | +      | +      |
| Kita-asuka cultivar   | R                     | R              | S      | +      | +      | +      | +      | +      |
| Kita-roman cultivar   | R                     | R              | S      | +      | +      | +      | +      | +      |
| Wasedainagon local variety | S            | S              | B      | -      | -      | -      | -      | -      |
| Akatsukidainagon cultivar | S          | S              | B      | -      | -      | -      | -      | -      |
| Akanedainagon cultivar | S                     | S              | B      | -      | -      | -      | -      | -      |
| Benidainagon cultivar | S                     | S              | B      | +      | -      | -      | -      | -      |
| Kamuidainagon cultivar | S                     | S              | B      | -      | -      | -      | -      | -      |
| Hokutodainagon cultivar | S                 | S              | B      | +      | -      | -      | -      | -      |
| Toyomidainagon cultivar | R                | R              | S      | +      | +      | +      | +      | +      |
| Tokiakari cultivar    | R                     | R              | S      | +      | +      | +      | +      | +      |
| Homaredainagon cultivar | R              | R              | S      | +      | +      | +      | +      | +      |
| Hokkaishiroshozu cultivar | S            | S              | B      | +      | -      | -      | -      | -      |
| Kitahotaru cultivar   | R                     | R              | S      | +      | +      | +      | +      | +      |

* R is resistance and ‘S’ is susceptibility.
* b co-dominant marker; ‘S’ is the ‘Syumari’ genotype and ‘B’ is the ‘Buchishoryukei-1’ genotype.
* c dominant marker to selectively amplify the genomic region from the BSR race 1 resistant cultivar ‘Syumari’.

from both ‘Syumari’ and ‘Buchishoryukei-1’. We could not amplify the region of Pg138-'Buchishoryukei-1'. The amplified regions sequenced in this study were submitted to the DDBJ (Accession no. from AB749324 to AB749332). Sequence characterized amplified region (SCAR) markers were developed to amplify the DNA fragment only from the resistant plants. In this study the melting temperature and secondary structure of all PCR primers were checked by using the DNA Calculator tool (http://www.sigma-genosys.com/calc/DNACalc.asp). The length of primers was 18 to 28 nucleotides, the GC content was 35 to 40% and the optimum melting temperature was 60 ± 5°C in order for annealing in PCR reactions to be performed at 55°C or 60°C. The PCR solution contained 0.2 mM each dNTP, 1.25 U Takara Ex Taq, 1× reaction buffer, 0.4 μM each primer and 50 ng template DNA in a total volume of 50 μl. The PCR protocol was as follows: 1 cycle of 95°C for 2 min; 35 cycles of 95°C for 30 s, 60°C or 55°C (in case of melting temperature was below 60°C and the sample did not amplify in 60°C) for 30 s, and 72°C for 2 min and 1 cycle of 72°C for 5 min.

**Selection of the marker closest to Pg118**

We genotyped 4278 F2 plants derived from 30 F1 plants of the crosses between ‘Syumari’ and ‘Buchishoryukei-1’ using the developed markers. The F2 plants that showed recombination between the markers were harvested. Six plants of the F3 progeny from the ten F2 plants selected were genotyped by the SCAR markers, and their BSR-resistance phenotype was evaluated using the inoculation test.
was designed as a common primer and primers Pg118-12 (5′-AGCTCGCCCACCTCATTT-3′). Primer Pg118-12 is specific to the susceptible bulk and two AFLP fragments (E-ACC/M-GGG-77, E-ACT/M-AGC-118) that were specific to the resistant bulk and two AFLP fragments (E-AAG/M-GAT-126, E-ATG/M-AAG-132) that were specific to the susceptible bulk were selected. The nucleotide sequence of E-ATG/M-AAG-132 and its flanking regions was identical to that of the E-ATG/M-GAG-138 region of the resistant parent. These two fragments therefore appeared to represent the same locus in each parent; we therefore excluded E-ATG/M-GAG-138 from our results. Finally, we developed five DNA markers based on differences in the nucleotide sequences of the AFLP fragments and their flanking regions between the resistant and susceptible parents (Table 2). Each marker was developed into a dominant selectable marker to amplify the resistant parent’s DNA and their flanking regions between the resistant and susceptible parents (Table 2). Each marker was developed into a dominant selectable marker to amplify the resistant parent’s DNA and their flanking regions between the resistant and susceptible parents (Table 2). Each marker was developed into a dominant selectable marker to amplify the resistant parent’s DNA and their flanking regions between the resistant and susceptible parents (Table 2).

### Results and Discussion

#### Development of DNA markers

Four AFLP fragments (E-ACC/M-GGG-77, E-ACT/M-AGC-118, E-ATG/M-GAG-138 and E-ACA/M-GAA-139) that were specific to the resistant bulk and two AFLP fragments (E-AAG/M-GAT-126, E-ATG/M-AAG-132) that were specific to the susceptiblebulk were selected. The nucleotide sequence of E-ATG/M-AAG-132 and its flanking regions was identical to that of the E-ATG/M-GAG-138 region of the resistant parent. These two fragments therefore appeared to represent the same locus in each parent; we therefore excluded E-ATG/M-GAG-138 from our results. Finally, we developed five DNA markers based on differences in the nucleotide sequences of the AFLP fragments and their flanking regions between the resistant and susceptible parents (Table 2). Each marker was developed into a dominant selectable marker to amplify the resistant parent’s DNA and their flanking regions between the resistant and susceptible parents (Table 2). Each marker was developed into a dominant selectable marker to amplify the resistant parent’s DNA and their flanking regions between the resistant and susceptible parents (Table 2). Each marker was developed into a dominant selectable marker to amplify the resistant parent’s DNA and their flanking regions between the resistant and susceptible parents (Table 2). Each marker was developed into a dominant selectable marker to amplify the resistant parent’s DNA and their flanking regions between the resistant and susceptible parents (Table 2). Each marker was developed into a dominant selectable marker to amplify the resistant parent’s DNA and their flanking regions between the resistant and susceptible parents (Table 2). Each marker was developed into a dominant selectable marker to amplify the resistant parent’s DNA and their flanking regions between the resistant and susceptible parents (Table 2). Each marker was developed into a dominant selectable marker to amplify the resistant parent’s DNA and their flanking regions between the resistant and susceptible parents (Table 2). Each marker was developed into a dominant selectable marker to amplify the resistant parent’s DNA and their flanking regions between the resistant and susceptible parents (Table 2). Each marker was developed into a dominant selectable marker to amplify the resistant parent’s DNA and their flanking regions between the resistant and susceptible parents (Table 2). Each marker was developed into a dominant selectable marker to amplify the resistant parent’s DNA and their flanking regions between the resistant and susceptible parents (Table 2).

#### Selection of the marker closest to Pga1

We identified 10 F2 plants that showed recombination for the developed DNA markers in the population of 4278 F2 individuals (Table 3). Therefore, the five markers were closely linked to one another and located near the BSR locus. Pg118 was the marker most closely linked to Pga1 based on the segregation of BSR resistance in the F3 progeny of the recombinant plants (i.e., it was the only marker present in all three resistant plants). We converted Pg118 into a codominant marker based on the differences in the nucleotide sequence of the AFLP fragment E-ACT/M-AGC-118 and its flanking regions between the resistant and susceptible parents. Primer Pg118-12 (5′-AGCTCGCCCACCTCATTT-3′) was designed as a common primer and primers Pg118-15 (5′-AAAATTGCTAGTGGAAATTGACTG-3′) and Pg118-21 (5′-GACATTTCCTTTCTCAGAAGATTACAG-3′) were designed as specific primers to amplify ‘Syumari’ and ‘Buchishoryukei-1’, respectively. This primer set was able to amplify DNA fragments from both resistant and susceptible F1 plants as well as other cultivars (Fig. 1). It is efficient if susceptible plants can be discarded prior planting. Therefore, the marker must be reliably amplified in susceptible genotypes and this was the case. Selection of resistant lines by means of MAS during early developmental stages of breeding would enable breeders to minimize their efforts on field selection. Han et al. (2005) reported a genetic linkage map of adzuki bean. It is important to map the developed markers into a public linkage group in future research.

#### General applicability of the developed markers

We used the 35 adzuki bean accessions and cultivars listed in Table 1 for genotyping using the developed markers, and 12 plants were evaluated their BSR resistance using the inoculation test.

| Marker | Primer Sense | Sequence (5′–3′) | reference sequences | Annealing temperature (°C) |
|--------|--------------|------------------|---------------------|--------------------------|
| Pg77   | Pg77F Forward | CCAAGTGAATAAACGAAACAGTAC | AB749324, AB749329 | 60 |
| Pg77R  | Reverse       | ATGGTGGTAAGTTGTTGGAAC | AB749325, AB749330 | 61 |
| Pg118  | Pg118F Forward | GGAATTCACTTCTCACAGTTATT | AB749325, AB749330 | 60 |
| Pg118R | Reverse       | AACATTTACTTAGCTACACTTTTGACAGA | AB749326, AB749330 | 64 |
| Pg126  | Pg126F Forward | GTGTGTTTATTTTGTATAGGAGCA | AB749326, AB749330 | 62 |
| Pg126R | Reverse       | CTTATATATAAGATAGATACACTCG | AB749327 | 65 |
| Pg138  | Pg138F Forward | GGATATATCAATGTCGTCCTTCGTC | AB749328, AB749330 | 56 |
| Pg138R | Reverse       | GTGTGACTGACGAACATTTAAATTAC | AB749328, AB749330 | 59 |
| Pg139  | Pg139F Forward | CTATCAACATTTTCCTCCTATACTAAG | AB749328, AB749330 | 62 |
| Pg139R | Reverse       | GGTATCCTGGAAGGAGTAAG | AB749328, AB749330 | 62 |

### General applicability of the developed markers in Japanese adzuki bean

We evaluated the resistance to BSR for 35 adzuki bean accessions and cultivars to confirm the general applicability of the developed markers into a public linkage group in future research.

| F2 plant number | SCAR marker genotype | BSR resistance
|-----------------|----------------------|---------------|
| No. 2983        | −−−−−              | Susceptible   |
| No. 1233        | −−−−+              | Susceptible   |
| No. 445         | −−+−+              | Susceptible   |
| No. 1393        | −−+++              | Susceptible   |
| No. 1900        | −−++−              | Susceptible   |
| No. 3163        | −−++−              | Susceptible   |
| No. 241         | −−−++              | Susceptible   |
| No. 435         | −−−−−              | Susceptible   |
| No. 1570        | −−−−−              | Susceptible   |
| No. 2909        | −−−−−              | Susceptible   |
| No. 3163        | −−++−              | Susceptible   |
| No. 241         | −−−++              | Susceptible   |

Recombinant F2 plants were selected from 4278 plants tested. a The BSR resistance was estimated based on the segregation in the progeny.
DNA markers linked to adzuki BSR resistance gene

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M 1 2 3 4 5 6 7 8 M

Fig. 1. Electrophoretic profiles of the Pg118 codominant marker in adzuki bean accessions/cultivars and segregants in the cross between ‘Symari’ and ‘Buchishoryukei-1’. M: 100 bp ladder size marker, 1: Syumari, 2: Buchishoryukei-1, 3: Kitano-otome, 4: Erimoshozu, 5: heterozygous genotype, 6: resistant genotype, 7: heterozygous genotype, 8: susceptible genotype.

(Okayama), ‘Maruba’ (Kari63), ‘Shochohin-10’, ‘Akamame’, ‘Acc259’ and eight cultivars bred from the cross combinations with any of the former three lines. All of the 13 lines and cultivars except for ‘Akamame’ and ‘Acc259’ possessed the same genotype as ‘Symari’ at four of the five dominant markers (Pg118, Pg126, Pg77 and Pg138); they possessed the same genotype as ‘Symari’ for the codominant Pg118 marker, whereas the remaining susceptible lines and cultivars did the genotype of ‘Buchishoryukei-1’. Chiba (1982) reported that ‘Kuroshozu’ (Okayama), ‘Maruba’ (Kari63) and ‘Shochohin-10’ were BSR-resistant and Chiba et al. (1987) reported that the BSR resistance derived from ‘Kuroshozu’ (Okayama) was controlled by a single dominant gene. The results obtained in the present study therefore suggest that ‘Maruba’ (Kari 63) and ‘Shochohin-10’ possess the same BSR resistance gene, Pgal, as ‘Kuroshozu’ (Okayama). In contrast, ‘Akamame’ and ‘Acc259’ had a different genotype from ‘Symari’ at Pg118 but were resistant to BSR in the inoculation test (Table 1). ‘Symari’ is resistant only to race 1 of C. gregata f. sp. adzukicola, whereas ‘Acc259’ is resistant to races 1 and 2 (Fujita et al. 2007, Kondo et al. 2005). ‘Acc259’ is therefore likely to carry different BSR resistance genes. Fujita et al. (2007) reported that the resistance to race 2 of BSR derived from ‘Acc259’ was controlled by a single dominant gene. The development of a DNA marker linked to the race 2 resistance gene in ‘Acc259’ would therefore be desirable in future research.

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Literature Cited

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