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Note

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 F_3 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-

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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 F_3 lines derived from a cross between *V. angularis* cv. 'Syumari' and cv. 'Buchishoryukei-1' to develop DNA markers. 'Syumari', 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 F_2 plants and their progeny derived from the same cross combination between 'Syumari' 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 8 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 F_3 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 F_3 plants. Each F_3 plant was selected from one F_3 line. AFLP analysis was carried out according to the method of Vos *et al.* (1995), with slight modification. Of the 1024 AFLP primers sets used in this study, the *Eco*RI 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 *Eco*RI (E) primers with three selective nucleotides and *Mse*I 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-500 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

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

Name	Accession or cultivar	BSR resistance[a]	SCAR marker genotype						Source of the resistance
			Pg118[b]	Pg139[c]	Pg118[c]	Pg126[c]	Pg77[c]	Pg138[c]	
Buchishoryukei-1	accession	S	B	–	–	–	–	–	
Kensaki	local variety	S	B	+	–	–	–	–	
Chagarawase	local variety	S	B	+	–	–	–	–	
Wase-maruba	local variety	S	B	–	–	–	–	–	
Wasedairyu	local variety	S	B	–	–	–	–	–	
Takahashi-wase	cultivar	S	B	–	–	–	–	–	
Kuroshozu (Okayama)	accession	R	S	+	+	+	+	+	
Maruba (Kari 63)	accession	R	S	+	+	+	+	+	
Shochohin-10	accession	R	S	–	+	+	+	+	
Akamame	accession	R	B	–	–	–	–	–	
Acc259	accession	R	B	–	–	–	–	–	
Takarashozu	cultivar	S	B	+	–	–	–	–	
Hikarishozu	cultivar	S	B	+	–	–	–	–	
Kotobukishozu	cultivar	S	B	–	–	–	–	–	
Sakaeshozu	cultivar	S	B	+	–	–	–	–	
Hayateshozu	cultivar	S	B	+	–	–	–	–	
Erimoshozu	cultivar	S	B	–	–	–	–	–	
Hatsuneshozu	cultivar	S	B	–	–	–	–	–	
Sahoroshozu	cultivar	S	B	+	–	–	–	–	
Akeno-wase	cultivar	S	B	–	–	–	–	–	
Kitano-otome	cultivar	R	S	+	+	+	+	+	Maruba (Kari 63)
Syumari	cultivar	R	S	+	+	+	+	+	Kuroshozu (Okayama)
Kita-asuka	cultivar	R	S	+	+	+	+	+	Maruba (Kari 63) or Kuroshozu (Okayama)
Kita-roman	cultivar	R	S	+	+	+	+	+	Maruba (Kari 63) or Shochohin-10
Wasedainagon	local variety	S	B	–	–	–	–	–	
Akatsukidainagon	cultivar	S	B	–	–	–	–	–	
Akanedainagon	cultivar	S	B	–	–	–	–	–	
Benidainagon	cultivar	S	B	+	–	–	–	–	
Kamuidainagon	cultivar	S	B	–	–	–	–	–	
Hokutodainagon	cultivar	S	B	+	–	–	–	–	
Toyomidainagon	cultivar	R	S	–	+	+	+	+	Shochohin-10
Tokiakari	cultivar	R	S	+	+	+	+	+	Kuroshozu (Okayama)
Homaredainagon	cultivar	R	S	+	+	+	+	+	Shochohin-10
Hokkaishiroshozu	cultivar	S	B	+	–	–	–	–	
Kitahotaru	cultivar	R	S	+	+	+	+	+	Maruba (Kari 63)

[a] '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 Pga1

We genotyped 4278 F_2 plants derived from 30 F_1 plants of the crosses between 'Syumari' and 'Buchishoryukei-1' using the developed markers. The F_2 plants that showed recombination between the markers were harvested. Six plants of the F_3 progeny from the ten F_2 plants selected were genotyped by the SCAR markers, and their BSR-resistance phenotype was evaluated using the inoculation test.

Table 2. SCAR markers developed in this study

Marker	Primer	Sense	Sequence (5′–3′)	reference sequences	Annealing temperature (C°)
Pg77	Pg77F	Forward	CCAAAGTAGAAAATAAGGAAACAGTAC	AB749324, AB749329	60
	Pg77R	Reverse	ATGGTTGTGAATTTATGTGTGAATC		62
Pg118	Pg118F	Forward	GGAATTCACTTCGAGTCATAATTC	AB749325, AB749330	61
	Pg118R	Reverse	AACATTTACTTAGCTACACTTTTGACA		60
Pg126	Pg126F	Forward	GTCGTTTATTTTGGTTATAGGAGCA	AB749326, AB749331	62
	Pg126R	Reverse	CCTGAATATATAAGATATATCCCGGGAC		64
Pg138	Pg138F	Forward	GGGATATATCAATGTGCTTTTGCTC	AB749327	65
	Pg138R	Reverse	GTGTGACTGACGAACTTTAATTTAC		59
Pg139	Pg139F	Forward	CTTACCAACATTTCTCTCTATACTAAG	AB749328, AB749332	56
	Pg139R	Reverse	GGATTCTTGGAATGATGGTGT		62

Primers were designed based on nucleotide sequences of a resistant cultivar 'Syumari' and a susceptible accession 'Buchishoryukei-1' for AFLP fragments polymorphic between the resistant and susceptible bulks and their flanking regions.

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.

Results and Discussion

Development of DNA markers

Four AFLP fragments (E-ACC/M-GGG-77, E-ACT/M-ACT-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 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 except for six nucleotides. These two fragments therefore appeared to represent the same locus in each parent; we therefore excluded E-ATG/M-AAG-132 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 ('Syumari') genotype.

Selection of the marker closest to Pga1

We identified 10 F_2 plants that showed recombination for the developed DNA markers in the population of 4278 F_2 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 F_3 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-ACT-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′-AAAATTGCTAGTGGAATTGACTG-3′) and Pg118-21 (5′-GCATTTCCTTTCCAGTAAAGATTACAG-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 F_3 lines 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 *el 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.

Table 3. Genotypes of recombinant F_2 plants for the five SCAR markers developed in this study and their BSR resistances

F_2 plant number	SCAR marker genotype					BSR resisntace[a]
	Pg139	Pg118	Pg126	Pg77	Pg138	
No. 241	+	−	−	−	−	Susceptible
No. 435	+	−	−	−	−	Susceptible
No. 1570	+	+	−	−	−	Heterozygous
No. 2909	+	+	−	−	−	Heterozygous
No. 1233	−	+	+	+	+	Heterozygous
No. 445	−	−	+	+	+	Susceptible
No. 1393	−	−	+	+	+	Susceptible
No. 1900	−	−	+	+	+	Susceptible
No. 3163	−	−	+	+	+	Susceptible
No. 2983	−	−	−	+	+	Susceptible

Recombinant F_2 plants were selected from 4278 plants tested.

[a] the BSR resistance was estimated based on the segregation in the progeny.

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 to the BSR resistance as tagging markers in Japanese adzuki bean. Of these, 13 showed the resistance to BSR (Table 1). These included 'Kuroshozu'

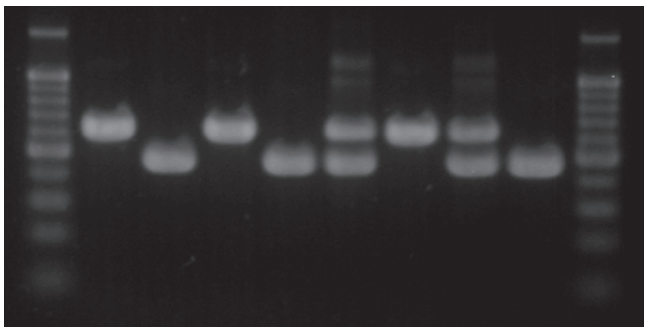

Fig. 1. Electrophoretic profiles of the Pg118 codominant marker in adzuki bean accessions/cultivars and segregants in the cross between 'Symari' and 'Buchishoryukei-1'. M: 100 bp ladder size marker, 1: Syumari, 2: Buchishoryukei-1, 3: Kitano-otome, 4: Erimoshozu, 5:heterozygous genotype, 6: resistant 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 'Syumari' at four of the five dominant markers (Pg118, Pg126, Pg77 and Pg138): they possessed the same genotype as 'Syumari' 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' (Kari 63) 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, *Pga1*, as 'Kuroshozu' (Okayama). In contrast, 'Akamame' and 'Acc259' had a different genotype from 'Syumari' at Pg118 but were resistant to BSR in the inoculation test (Table 1). 'Syumari' 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.

Acknowledgments

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Literature Cited

Chiba, I. (1982) Breeding for resistance to brown stem rot (BSR) in adzuki beans. Bull. Hokkaido Pref. Agric. Exp. Stn. 48: 56–63.

Chiba, I., T. Narikawa, K. Murata and T. Adachi (1987) Breeding for resistance to brown stem rot (BSR) in adzuki beans. III. The inheritance of resistance to BSR and effect of its introduction. Bull. Hokkaido Pref. Agric. Exp. Stn. 56: 1–7.

Fujita, S. (2007) Studies on the breeding of adzuki bean cultivars resistant to adzuki bean brown stem rot (BSR) and phytophthora stem rot (PSR). Hokkaido Agric. Exp. Stn., Naganuma, Japan. Report 115.

Fujita, S., K. Murata, H. Shimada, S. Aoyama, I. Chiba, I. Matsukawa, S. Shirai, T. Miura and N. Kondo (2002) A new adzuki bean cultivar Syumari with soil-borne disease resistance and excellent processing quality. Bull. Hokkaido Pref. Agric. Exp. Stn. 82: 31–40.

Fujita, S., N. Kondo, H. Shimada, K. Murata and S. Naito (2007) Re-evaluation and selection of adzuki beans (*Vigna angularis*) to breed cultivars resistant to a new race of *Phialophora gregata* f. sp. *adzukicola*, the causal agent of adzuki bean brown stem rot (BSR). Breed. Res. 9: 87–95.

Han, O.K., A. Kaga, T. Isemura, X.W. Wang, N. Tomooka and D.A. Vaughan (2005) A genetic linkage map for azuki bean [*Vigana angularis* (Willd.) Ohwi & Ohashi]. Theol. Appl. Genet. 111: 1278–1287.

Harrington, T.C. and D.L. McNew (2003) Phylogenetic analysis places the *Phialophora*-like anamorph genus *Cadophora* in the Helotiales. Mycotaxon 87: 141–151.

Kobayashi, K., H. Yamamoto, H. Negishi and A. Ogoshi (1991) *Formae specialis* differentiation of *Phialophora gregata* from adzuki bean and soybean in Japan. Ann. Phytopathol. Soc. Jpn. 57: 225–231.

Kondo, N., S. Fujita, K. Murata and A. Ogoshi (1998) Detection of two races of *Phialophora gregata* f. sp. *adzukicola*, the causal agent of adzuki bean brown stem rot. Plant Dis. 82: 928–930.

Kondo, N., A. Notsu, S. Naito, S. Fujita and H. Shimada (2004) Distribution of *Phytophthora vignae* f. sp. *adzukicola* races in adzuki bean fields in Hokkaido, Japan. Plant Dis. 88: 875–877.

Kondo, N., K. Nakazawa, S. Fujita, H. Shimada and S. Naito (2005) New virulent race of *Phialophora gregata* f. sp. *adzukicola* associated with continuous cultivation of adzuki bean cultivar Acc259. J. Gen. Plant Pathol. 71: 360–363.

Kondo, N., H. Shimada and S. Fujita (2009) Screening of cultivated and wild adzuki bean for resistance to race 3 of *Cadophora gregata* f. sp. *adzukicola*, cause of brown stem rot. J. Gen. Plant Pathol. 75: 181–187.

Kondo, N. and N. Tomooka (2012) New sources of resistance to *Cadophora gregata* f. sp. *adzukicola* and *Fusarium oxysporum* f. sp. *adzukicola* in *Vigna* spp. Plant Dis. 96: 562–568.

Michelmore, R.W., I. Paran and V. Kesseli (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA 88: 9828–9832.

Murray, M.G. and W.F. Thompson (1980) Rapid isolation of high molecular weight DNA. Nucleic Acids Res. 8: 4321–4326.

Ochman, H., A.S. Gerber and D.L. Hartl (1988) Genetic applications of an inverse polymerase chain reaction. Genetics: 621–623

Takeda, A., T. Suzuki, T. Takeuchi, S. Fujita, H. Shimada, N. Kondo and S. Naito (2006) Screening of adzuki bean germplasm for adzuki bean brown stem rot resistance gene using DNA markers. Jpn. J. Phytopathol. 72: 85.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper *et al.* (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407–4414.