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Screening of cultivated and wild adzuki bean for resistance to race 3 of *Cadophora gregata* f. sp. *adzukicola*, cause of brown stem rot

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Abstract

Two diseases of adzuki bean, brown stem rot (BSR, caused by *Cadophora gregata* f. sp. *adzukicola*) and adzuki bean Fusarium wilt (AFW, caused by *Fusarium oxysporum* f. sp. *adzukicola*), are serious problems in Hokkaido and have been controlled using cultivars with multiple resistance. However, because a new race of BSR, designated race 3, was identified, sources of parental adzuki bean for resistance to race 3 were needed. Therefore, we examined 67 cultivars and lines of cultivated and wild adzuki bean maintained at the Tokachi Agricultural Experiment Station using a root-dip inoculation method. Consequently, nine adzuki bean cultivars, one wild adzuki bean accession and 30 lines (including two lines resistant to all the three races of BSR and AFW) were confirmed to be resistant or tolerant to race 3 of BSR, and we found a cultivar Akamame as well as a wild adzuki bean Acc2515 to be a new source for a resistance gene to the race 3. This cultivar also holds promise as a source of resistance against other races of BSR and AFW.

Key Words: adzuki bean, brown stem rot, *Cadophora gregata* f. sp. *adzukicola*, *Fusarium oxysporum* f. sp. *adzukicola*, Fusarium wilt, race, soilborne fungi

Brown stem rot (BSR) of adzuki bean [*Vigna angularis* (Willd.) Ohwi et Ohashi] caused by *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), a soilborne fungus, is a serious problem in Hokkaido, Japan (Fujita 2004). Adzuki bean Fusarium wilt (AFW) caused by *Fusarium oxysporum* Schlechtendal: Fr. emend. Snyder & Hansen f. sp. *adzukicola* Kitazawa & Yanagita (Kitazawa and Yanagita 1989), another soilborne fungus, is often observed in the western part of Hokkaido (Fujita 2004; Kondo and Kodama 1989). Both diseases, along with *Phytophthora* stem rot caused by *Phytophthora vignae* Purss f. sp. *adzukicola* Tsuchiya, Yanagawa et Ogoshi (Tsuchiya et al. 1986) are economically important constraints to adzuki bean production (Harada and Kondo 2009; Kondo et al. 2004). Both diseases are characterized by wilting and a reddish brown discoloration of vascular and pith tissues of the stem and the petiole that often occur in conjunction with leaf chlorosis or necrosis (Kondo and Kodama 1989; Narita et al. 1971). Although phytotoxic gregatins produced by *C. gregata* f. sp. *adzukicola* were previously considered to be associated with the appearance of these symptoms (Kobayashi and Ui 1977), Tanaka et al. (2007) found that these toxins were unlikely to be associated with pathogenicity. Vein necrosis and wilting of leaves are evident in AFW 3 to 4 weeks after inoculation with *F. oxysporum* f. sp. *adzukicola*, while external symptoms such as defoliation without vein necrosis are apparent for BSR 6 weeks or later after inoculation with *C. gregata* f. sp. *adzukicola*. In both cases, these two pathogens invade the root tissues of young plants and then spread upwards in the plant vascular and pith tissues via mycelia and conidia (Kondo and Kodama 1989; Narita et al. 1971) to cause wilting of susceptible plants and to reduce yields of adzuki bean.

The multiple resistance of adzuki bean cultivars Kita-no-otome and Syumari has contributed greatly to the control of the two diseases, especially of AFW (Fujita et al. 1995, 2002; Kondo et al. 1998, 2002). Although race 2 of BSR, that is virulent to these two cultivars, was discovered in 1995 (Kondo et al. 1998; 2002), several cultivated and wild adzuki bean accessions resistant to both races 1 and 2 of BSR were selected as potential sources of BSR resistance for breeding programs (Fujita et al., 2007). However, Acc259, one of the accessions used as a gene donor for a pyramid-type resistance in new lines, was completely susceptible to a new race (race 3) (Kondo et al. 2005). Thus, resistance to race 3 also needed to be considered in breeding for multiple resistances in adzuki bean.

We hypothesized that a considerable number of cultivars and lines of adzuki bean should be resistant to race 3; these were identified easily because Syumari and Kita-no-otome have been determined to be resistant to both races 1 and 3 (Kondo et al. 2005), a pleiotropic resistance gene against both races, and predicted in both cultivars by specific DNA markers. The same markers for the resistance gene were indeed identified in several other cultivars (Takeda et al. 2006). To further evaluate our hypothesis and to find more sources of resistance genes, we evaluated germplasm accessions kept at the Tokachi Agricultural Experiment Station (TAES; Memuro, Japan) for their resistance to race 3 of BSR.

Plant materials

Sixty-six adzuki bean cultivars or lines and one accession (Acc2515 kindly given to the TAES by Dr. H. Yamaguchi, Osaka Prefecture University, Sakai, Japan) of wild adzuki bean, *V. angularis* var. *nipponensis*, were used in the present study (Table 1). The seeds were preserved and propagated at TAES. Seedlings were grown in plastic

containers (15 × 20 × 5 cm) with sterilized vermiculite for about 10 to 14 days in greenhouse, then the roots were washed gently with running tap water before inoculation with the pathogens.

Fungal isolates and preparation of inocula

The following isolates of *C. gregata* were used and deposited in NIAS Genebank (National Institute of Agrobiological Science, Tsukuba, Japan): T96-1 (race 1, accession MAFF 241056), T96-5 (race 2, MAFF 241057), and T650 (race 3, MAFF 241055) (Kondo et al. 1998; 2005). Inocula for *C. gregata* were produced in liquid V8 juice medium (200 ml V8 juice containing 2 g CaCO₃ was centrifuged at 1,400 × *g* for 15 min and the supernatant was diluted to 1 L with distilled water) at 25°C on a reciprocal shaker (TB-128, Takasaki Scientific Instrument, Kawaguchi, Japan) at 120 oscillations/min. After 3 weeks, mycelia and budcells were collected by filtering through filter paper (Whatman No.1), then washed by suspending in distilled water followed by centrifugation. The fungal pellets were homogenized in distilled water by a homogenizer (10,000 rpm for 3 min; AN-5, Shin-nihonseiki, Tokyo, Japan). The concentration of mycelial fragments and bud cells was determined using a hemocytometer; mycelial fragments of all sizes were included in counts. Blended cultures were then diluted with distilled water to a concentration of 10⁷ propagules per milliliter. Isolate 96-3K2 of *F. oxysporum* f. sp. *adzukicola*, identified as race 3 and having the widest host range (Kondo et al. 1989), was deposited in the NIAS Genebank (accession MAFF 241054). Inoculum was produced in potato sucrose broth medium (Difco, Lawrence, Kansas, USA) with 2% yeast extract (Difco) at 25°C on a reciprocal shaker at 120 oscillations/min. After 5 days of incubation, bud cells were collected by centrifugation at 1,400 × *g* for 10 min and were washed by suspending in distilled water

followed by centrifugation. The concentration was adjusted with a hemocytometer to 10^6 bud cells /ml distilled water.

Screening for resistance

The roots of 12 seedlings of each cultivar or line were dipped into a suspension (50 ml) of each inoculum for 12 h. The seedlings were removed from the inoculum and placed into a depression 2–3-cm deep in a sterilized mixed soil (1:1, v/v, vermiculite/ Pot-ace; Katakura Chikkarin K. K., Tokyo, Japan) filling an 18-cm diameter plastic pot. The remaining inoculum was then poured over the roots of seedlings to maximize coverage, and the roots were covered with the sterilized mixed soil to a level —3 cm below the primary leaves. The test was conducted twice in all combinations except for lines Tokei 934 and Tokei 935, for which an additional test was conducted. Nighttime low and daytime high temperatures in a greenhouse during these tests were 15/32°C. Plants received supplemental lighting from metal halide sodium lamps (400 W) to maintain a 16-h photoperiod. For evaluation of BSR and AFW incidence, the reaction of individual plants was scored 8 weeks post-inoculation according to a 0–3 disease severity index (Table 1).

Four response groups were categorized as in Table 1: (A) susceptible to all pathogens tested, (B) susceptible to BSR race 2 but resistant to the other pathogens including BSR race 3, (C) susceptible to BSR race 3 but resistant to the other pathogens, and (D) resistant to all pathogens tested. Of the 67 cultivars and lines tested, 40 were classified as resistant to BSR race 3 (36 cultivars and lines in the group B, three cultivars and lines and one wild adzuki bean accession in group D), while 26 in group A and one in group C, Acc259, were susceptible. Thus, a considerable number of cultivars and lines in the group B resistant to race 1 also seemed to be resistant to race 3. This

result may be reflected by the fact that the frequencies of races 1 and 3 were much lower than that of race 2 in the BSR pathogen population of the experiment field of TAES where a large number of genotypes resistant to race 1 had been cultivated for more than 20 years (Kondo et al. 2005). A putative pleiotropic resistance gene *Pga1* in Syumari that is resistant to races 1 and 3 of BSR and AFW on a locus located in the third linkage group also has been predicted (Han et al. 2005; Takeda et al. 2007). Because of the presence of specific DNA markers for the resistant gene (Takeda et al. 2006), eight cultivars in the group B [Kita-no-otome, Syumari, Toki-akari, Toyomi-dainagon, Kita-hotaru, Kuro-shozu (Okayama), Emba (Kari63), and Shochohin-10] were presumed to possess the same putative *Pga1* gene. Hence, these cultivars should be sources of resistance genes to BSR race 3 as well as BSR race 1 and AFW. However, further genetic analysis of each cultivar in group B is needed to identify any resistance genes.

Meanwhile, cultivar Akamame and wild adzuki bean Acc2515 were eventually determined to be completely resistant to all pathogens including BSR race 3, although resistance to AFW in Akamame and to all three races of BSR in Acc2515 have been reported (Kondo and Kodama 1989; Kondo et al. 2005). Both cultivars Hatsune-shozu and Akeno-wase in group A, which were originally derived from Akamame, were previously developed BSR-tolerant cultivars (Adachi et al. 1988; Shimada et al. 1992), but we categorized them as susceptible in the present test because obvious foliar or stem symptoms were observed in artificial inoculation tests with races 1 and 3 of BSR. Even though a major gene dominated resistance to BSR in Akamame, this cultivar may have some additional minor or modifier genes with partial resistance effects (Chiba et al. 1987; Fujita et al. 2007) that differ from the dominant gene of Kita-no-otome or Syumari (Fujita et al. 1995; 2002). Specific DNA markers for the

resistance gene (*Pga1*) to races 1 and 3 that were shown in the eight cultivars in group B described were not found in Akamame (Takeda et al. 2006). In light of these results, Akamame may have new genes for resistance to race 3 of BSR. Further analysis of resistance genes in Akamame and Acc2515 may reveal a novel mechanism of resistance to BSR and AFW.

Lines Tokei 934 and Tokei 935, which were originally derived from a cross of Syumari and Acc259, had practical resistance against all races of BSR and AFW in this study. Those lines were accordingly expected to be candidates for new cultivars with multiple resistance conferred by genes from Acc259 and Syumari against BSR races 2 and 3, respectively. However, the genetic segregation of resistance to races 2 and 3 of BSR in Tokei 934 and fixation of only *Pga2* (a putative gene for resistance to race 2 of BSR in Acc259) in Tokei 935 were determined using specific DNA markers for resistance genes, *Pga1* and *Pga2* (T. Takeuchi, personal communication). Indeed, discolored vascular bundles were observed in a few plants of these two lines when inoculated with race 3 of BSR, but no external symptoms, defoliation or blight, developed on these plants in the present study. Therefore, although the contradiction between the results of identification of resistance genes and the inoculation tests was not resolved, both lines should be regarded at this time as substantially resistant to BSR and AFW. Further investigations are needed to clarify the genetics of resistance of these lines. Besides, adzuki bean varieties resistant to all pathogens were likely to be rare. As well as the work of Fujita et al. (2007), in which in a collection of wild adzuki beans were screened for sources of resistance to race 2 of BSR, further screening of wild adzuki beans from various locations is necessary in order to develop cultivars with multiple resistance.

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Table 1. Responses of *Vigna angularis* to *Cadophora gretata* f. sp. *adzukiicola* and *Fusarium oxysporum* f. sp. *adzukiicola*

Group	Cultivar /Line	Origin	Disease severity index											
			<i>C. gregata</i>									<i>F. oxysporum</i>		
			T96-1 (race 1)			T96-5 (race 2)			T650 (race 3)			96-3K2 (race 4)		
			1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
A	Buchi-syoryu no.1	Hokkaido, Japan	1.5	2.4	-	S	2.0	2.3	-	S	1.8	1.0	-	S
	Kensaki	Hokkaido, Japan	2.0	2.3	-	S	2.7	2.9	-	S	1.7	2.3	-	S
	Chagarawase	Hokkaido, Japan	1.7	2.9	-	S	2.0	3.0	-	S	2.0	3.0	-	S
	Wasemaruba	Hokkaido, Japan	2.0	3.0	-	S	3.0	3.0	-	S	2.0	2.1	-	S
	Wasetairyu	China	2.0	3.0	-	S	2.0	3.0	-	S	2.0	2.9	-	S
	Takahashiwase	Maruba × Buchi-syoryu No.1	2.0	3.0	-	S	2.0	3.0	-	S	2.0	3.0	-	S
	Wasetairyu no.1	Pure line of Wasetairyu	2.0	3.0	-	S	2.0	3.0	-	S	2.8	3.0	-	S
	Emba no.1	Pure line of Emba (Tsukigata)	2.0	2.9	-	S	1.6	3.0	-	S	3.0	3.0	-	S
	Takara-syozu	Mass selection from Syozu (W45)	2.0	3.0	-	S	2.0	3.0	-	S	1.7	2.9	-	S
	Hikari-syozu	Chagarawase × Wasetairyu No.1	2.0	3.0	-	S	2.0	3.0	-	S	1.7	3.0	-	S
	Kotobuki-syozu	Noto-shozu × Wasetairyu No.1	2.0	2.9	-	S	2.0	2.9	-	S	2.0	2.4	-	S
	Sakae-syozu	3407 × Wasetairyu No.1	2.0	2.3	-	S	2.0	3.0	-	S	1.7	2.9	-	S
	Hayate-syozu	Takara-syozu × Buchi-syoryu No.1	1.6	1.0	-	S	1.0	2.7	-	S	2.0	2.9	-	S
	Erimo-syozu	Kotobuki-syozu × Toiku 77	2.0	2.0	-	S	2.0	2.3	-	S	1.7	2.1	-	S
	Hatsune-syozu	Hayate-syozu × Akamame	1.4	0.8	-	S-I	0.6	1.2	-	S-I	2.0	2.3	-	S
	Sahoro-syozu	Akane-dainagon × Cyugokuzairai No.1	2.0	2.6	-	S	1.9	3.0	-	S	1.4	2.3	-	S
	Akeno-wase	Tokei 276 × Toiku 106	1.0	0.6	-	S-I	1.4	1.2	-	S	2.0	2.9	-	S
	wase-dainagon (Mamakotou)	Hokkaido, Japan	2.0	1.0	-	S	2.0	3.0	-	S	2.0	1.5	-	S
	Akatsuki-dainagon	Noto-syozu × Wasetairyu No.1	2.0	2.2	-	S	2.1	3.0	-	S	2.0	2.4	-	S
	Akane-dainagon	Noto-syozu × Wasetairyu No.1	2.0	3.0	-	S	2.0	2.0	-	S	1.7	3.0	-	S
	Beni-dainagon	Toiku 85 × Seigen-harushozu	2.0	2.8	-	S	2.6	3.0	-	S	1.9	2.2	-	S
	Kamui-dainagon	Toiku 106 × Tokei 207	2.0	2.8	-	S	2.0	2.4	-	S	1.7	3.0	-	S
	Hokuto-dainagon	Toiku 113 × Toiku 80	2.0	3.0	-	S	1.6	2.5	-	S	1.4	2.8	-	S
	Hokkai-shiro-syozu	Shiro-syozu (Kawanishi) × Chagarawase	2.0	3.0	-	S	2.0	2.5	-	S	1.9	2.9	-	S
	Toiku 126	Tokei 207 × Mikamo-dainagon	2.0	3.0	-	S	1.8	2.8	-	S	2.0	3.0	-	S
	Acc1218	Kochi, Japan	2.0	2.5	-	S	0.7	2.9	-	S	2.8	1.4	-	S

B	Kuro-syozu (Okayama)	Okayama, Japan	0.0	0.0	-	R	0.8	1.0	-	S-I	0.0	0.0	-	R	0.0	0.0	-
	Emba (Kari63)	Akita, Japan	0.0	0.0	-	R	2.0	2.3	-	S	0.0	0.0	-	R	0.0	0.0	-
	Syochohin 10	Nagano, Japan	0.0	0.0	-	R	2.0	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Kita-no-otome	Erimo-syozu × 2025	0.0	0.0	-	R	2.0	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Syumari	Tokei 494 × Tokei 486	0.0	0.0	-	R	2.0	3.0	-	S	0.0	0.0	-	R	0.0	0.4	-
	Tokiakari	Tokei 617 × Toiku 122	0.0	0.0	-	R	1.7	2.9	-	S	0.0	0.0	-	R	0.0	0.0	-
	Toyomi-dainagon	92089 × Tokei 564	0.0	0.0	-	R	2.0	2.6	-	S	0.0	0.0	-	R	0.0	0.0	-
	Kitahotaru	93142(F6) × Tokei 590	0.0	0.0	-	R	2.0	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Toiku 123	Toiku 97 × Syochohin-10	0.0	0.0	-	R	1.4	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Toiku 147	Toiku 137 × Toiku 138	0.0	0.0	-	R	2.0	2.8	-	S	0.0	0.0	-	R	0.0	0.2	-
	Toiku 148	Toiku 137 × Toiku 138	0.0	0.0	-	R	2.3	2.8	-	S	0.0	0.0	-	R	0.0	0.2	-
	Toiku 149	Toiku 137 × Tokei 651	0.0	0.0	-	R	2.0	2.5	-	S	0.0	0.0	-	R	0.0	0.2	-
	Toiku 150	Toiku 137 × Tokei 651	0.0	0.0	-	R	2.0	3.0	-	S	0.0	0.0	-	R	0.0	0.2	-
	Toiku 151	Tokei 691 × Toiku 142	0.0	0.0	-	R	2.0	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Toiku 152	Tokei 656 × Tokei 726	0.0	0.0	-	R	2.0	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Toiku 153	Tokei 701 × Tokei 697	0.0	0.0	-	R	2.3	2.2	-	S	0.0	0.0	-	R	0.0	0.0	-
	Toiku 154	Tokei 701 × Tokei 697	0.0	0.0	-	R	2.0	2.2	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 864	9429F4 × Toiku 140	0.0	0.0	-	R	2.0	2.3	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 889	Tokei 741 × Buchisyoryu No.1	0.0	0.0	-	R	2.2	2.3	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 890	Toiku 141 × Tokei 732	0.0	0.0	-	R	1.7	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 898	Erimo-syozu × Toiku 140	0.0	0.0	-	R	2.0	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 901	Erimo-syozu × Toiku 141	0.0	0.0	-	R	2.3	2.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 904	Hokuto-dainagon × 9420-24	0.0	0.0	-	R	2.0	2.9	-	S	0.0	0.1	-	R	0.0	0.0	-
	Tokei 906	9426-71(F6) × Tokei 726	0.0	0.0	-	R	2.6	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 912	9501-21(F6) × 9504-24(F6)	0.0	0.0	-	R	1.8	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 913	9501-21(F6) × 9504-24(F6)	0.0	0.0	-	R	2.4	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 914	9501-21(F6) × 9504-24(F6)	0.0	0.0	-	R	3.0	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 915	9501-21(F6) × 9504-24(F6)	0.0	0.0	-	R	3.0	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 916	9501-21(F6) × 9414-14(F6)	0.0	0.0	-	R	2.4	2.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 917	9501-21(F6) × 9414-14(F6)	0.0	0.0	-	R	2.3	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 918	9501-21(F6) × 9414-14(F6)	0.0	0.0	-	R	2.1	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 919	9501-21(F6) × 9414-14(F6)	0.0	0.0	-	R	2.1	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 920	9501-21(F6) × 9414-14(F6)	0.0	0.0	-	R	1.7	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-

	Tokei 921	9501-21(F6) × 9414-14(F6)	0.0	0.0	-	R	1.6	1.2	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 922	9501-21(F6) × 9414-14(F6)	0.0	0.0	-	R	2.4	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 925	Toiku 746 × Beni-dainagon	0.0	0.0	-	R	2.0	3.0	-	S	0.0	0.0	-	R	0.0	0.0	-
	Tokei 936	Tokei 794 × Toiku 140	0.0	0.0	-	R	1.7	2.0	-	S	0.0	0.1	-	R	0.0	0.3	-
C	Acc259	Ehime, Japan	0.0	0.0	-	R	0.0	0.0	-	R	2.0	1.4	-	S	0.0	0.0	-
D	Akamame	Korea	0.0	0.0	-	R	0.0	0.0	-	R	0.0	0.0	-	R	0.0	0.0	-
	Acc2515	Ishikawa, Japan	0.0	0.0	-	R	0.0	0.0	-	R	0.0	0.0	-	R	0.0	0.3	-
	Acc1238	Niigata, Japan	0.0	0.0	-	R	0.0	0.3	-	R	0.0	0.0	-	R	0.0	0.0	-
	Tokei 934	9609-1(F6) × 9930-5(F5)	0.0	0.0	0.0	R	0.0	0.0	0.0	R	0.3	0.0	0.9	R-I	0.0	0.0	0
	Tokei 935	9609-1(F6) × 9930-5(F5)	0.0	0.0	0.0	R	0.0	0.0	0.0	R	0.0	0.3	0.5	R-I	0.0	0.0	0

^a Disease severity of brown stem rot caused by *C. gregata* was assessed 8 weeks after inoculation in greenhouse experiments as the mean disease severity index (DSI) with a scale where 0 = no symptoms, 1 = a slight vascular discoloration without foliar symptoms, 2 = foliar symptoms or severe vascular discolorations (above the first node or in petioles), and 3 = death. Plants with a DSI < 0.5 were rated resistant (R), plants with 0.5 ≤ a DSI < 1.0 were regarded as intermediate (I) and plants with a DSI ≥ 1.0 were considered as susceptible (S). For Fusarium wilt, the reaction of individual plants was scored 8 weeks after inoculation in greenhouse experiments using a disease severity rating (DSR) of 1 to 10³, where 1 = healthy, no external symptoms and no vascular discoloration; 10 = plants with vascular discoloration, but no external symptoms; 10² = plants with leaf necrosis, stunting or wilting; 10³ = dead. DSI was calculated from the formula: DSI = log [mean (DSR)]. Plants with DSI ≤ 1.0 were rated resistant (R) and plants with DSI > 1.0 were intermediate (I) or susceptible (S).