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FUNGAL DISEASE

Short communication

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New race of *Phytophthora vignae* f. sp. *adzukicola*, the causal agent of Phytophthora stem rot of the adzuki bean

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Abstract A new race of *Phytophthora vignae* f. sp. *adzukicola*, designated race 4, is reported from central and western Hokkaido, Japan. The isolates obtained from diseased plants of a new cultivar, cv. Syumari, which is resistant to races 1, 2, and 3, were determined to be a new race by the pathogenic reaction on a set of differential adzuki bean cultivars (cv. Erimo-shozu, cv. Kotobuki-shozu, cv. Noto-shozu, cv. Urasa-shimane, and cv. Syumari).

Key words Phytophthora stem rot · *Phytophthora vignae* f. sp. *adzukicola* · Race · *Vigna angularis*

There has been an epidemic of Phytophthora stem rot of the adzuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi] caused by *Phytophthora vignae* Purss f. sp. *adzukicola* Tsuchiya, Yanagawa et Ogoshi on Hokkaido, Japan, especially in the central and western regions of the island (Kitazawa et al. 1978). Initially, the disease was suspected to be caused by a strain of *P. vignae* Purss, the causal agent of Phytophthora stem rot of cowpea [*Vigna ungiculata* (L.) Walp.] (Kitazawa et al. 1979). Kitazawa et al. (1979) and Tsuchiya et al. (1986), however, showed that the pathogen causing Phytophthora stem rot of adzuki bean was another pathogenic forma specialis of *P. vignae* consisting of three physiologic races: race 1 (virulent to cv. Takara-shozu but not to cv. Kotobuki-shozu or cv. Noto-shozu); race 2 (virulent to cv. Takara-shozu and cv. Kotobuki-shozu but not to cv. Noto-shozu); and race 3 (virulent to all three cultivars). The extensive survey of the distribution of the races during 1977–1979, showed that races 1 and 3 were predominant and that race 2 had low frequency on Hokkaido Island. In a recent study, the frequency of the races was found to be similar to the earlier result, although race 2 was not observed (Makino et al. 1997).

To control the disease, a new resistant cultivar, cv. Syumari, was developed from cv. Urasa-shimane, which is resistant to races 1, 2, and 3. Phytophthora stem rot was observed on cv. Syumari in the test fields used to evaluate productivity in central and western Hokkaido in 1999. The present study was undertaken to determine if a new race of *Phytophthora vignae* f. sp. *adzukicola* has developed.

Phytophthora species were isolated on cornmeal agar plates from lesions in which oospores were observed profusely on cv. Syumari and cv. Erimo-shozu plants from fields in Oiwake, Kyogoku, and Haboro in 1999. Six representative isolates – Pv-o1, Pv-o2, Pv-o3 (Oiwake), Pv-h1, Pv-h2 (Haboro), and Pv-k12–1 (Kyogoku) – were cultured on V8 agar (Ishiguro and Ui 1981); and oospores, oogonia, and antheridia were then examined morphologically under a microscope. Zoosporangia were also observed after preparing mycelia by culturing each isolate in pea broth for 10 days. They were washed twice with natural mineral water (Ca^{2+} 27.2 mg/l, Ka^{+} 4.9 mg/l, Mg^{2+} 9.40 mg/l, Na^{+} 17.8 mg/l; pH 7.9) in a petri dish, submerged in mineral water, and incubated for 1 or 2 days (Eye et al. 1978). *P. vignae* f. sp. *adzukicola* race 3 (IFO30613 originally isolated in Asahikawa, Hokkaido in 1977 (Tsuchiya et al. 1986)) was used for comparison. All of the *Phytophthora* isolates examined were homothallic; oogonia were smooth-walled and measured 27.3–39.7 μm in diameter; oospores were spherical, markedly aplerotic (24.8–34.7 μm in diameter), and turned pale yellow; and antheridia were amphigynous (average 15.2 × 14.7 μm). Many hyphal swellings formed. The zoosporangia were globose or ovoid in shape and nonpapillate; they were 27.4–56.8 μm long × 13.7–35.0 μm wide. The sizes of the organs were similar to those recorded elsewhere (Kitazawa et al. 1978). Morphologically, the isolates were identified as *P. vignae* Purss (1957).

Isolates Pv-o1, Pv-o3, and IFO30613 were subjected to a host-range test using legumes (adzuki bean, cowpea, com-

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Table 1. Pathogenicity of isolates of *P. vignae* f. sp. *adzukicola* to adzuki bean cultivars

Isolate[a]	Disease severity index[b]					Race
	Erimo-shozu	Kotobuki-shozu	Noto-shozu	Urasa-shimane	Syumari	
Oiwake						
Pv-o1	2.8 S	2.2 S	2.0 S	2.5 S	2.3 S	4
Pv-o2	2.8 S	2.2 S	2.8 S	2.4 S	3.0 S	4
Pv-o3	3.0 S	2.3 S	2.8 S	2.6 S	3.0 S	4
Haboro						
Pv-h1	2.2 S	1.6 S	1.9 S	1.3 S	2.2 S	4
Pv-k12-1	1.7 S	1.3 S	1.3 S	1.6 S	1.8 S	4
Kyogoku						
Pv-k11	1.9 S	0.3 R	0.1 R	0.1 R	0.1 R	1
Asahikawa						
IFO30613	1.7 S	1.8 S	1.3 S	0.0 R	0.1 R	3

Tests were repeated twice for isolates Pv-o2 and Pv-o3 and three times for the other isolates
S, susceptible; R, resistant
[a] Pv-o1, Pv-o2, Pv-o3, Pv-h1, and Pv-k12-1 were isolated from cv. Syumari in 1999. Pv-k11 and IFO30613 were isolated from cv. Erimo-shozu in 1999 and from cv. Takara-shozu in 1977
[b] Disease severity was assessed 2 weeks after transplanting as the mean disease severity index (DSI) with the following scale: 0, no symptoms; 1, a slight or restricted lesion on hypocotyls; 2, an expanding lesion on hypocotyls; and 3, death. Reaction class was based on the DSI scale: <1.0, resistant (R); ≥ 1.0, susceptible (S)

mon bean, soybean). Mycelia (1 g, fresh weight) from 2-week-old cultures of each isolate grown in pea broth at 25°C was homogenized (Nissei AM-5) at 5000 rpm for 3 min with 100 ml of sterilized distilled water. Seven- to ten-day-old seedlings grown in sterilized vermiculite were washed gently with running tap water before inoculation. Nine to eleven seedlings of each legume plant were soaked in the suspension of each inoculum for 12 h. The seedlings were then transplanted into a mixed (1:1, v/v) soil (vermiculite/Pot Ace; Katakura Chikkarin K.K., Tokyo, Japan) in plastic pots (18 cm in diameter). Three weeks after transplanting under greenhouse conditions (12°–25°C), the incidence of disease (dead or alive) was evaluated. All of the isolates were pathogenic only to adzuki bean (cv. Erimo-shozu, 63%–91% plants dead) but not to cowpea (cv. Akatane-sanjaku and cv. Kegon-no-taki), common bean [*Phaseolus vulgaris* L., cv. Ohfuku], or soybean [*Glycine max* (L.) Merr., cv. Wase-midori and cv. Yuki-musume]. These results suggest that the isolates had pathogenicity specific to adzuki bean. Based on their morphological and pathological characteristics, the isolates were thought to be *P. vignae* f. sp. *adzukicola* (Tsuchiya et al. 1986).

The five cultivars of adzuki bean listed in Table 1 were inoculated with isolates Pv-o1, Pv-o2, Pv-o3, Pv-h1, Pv-k11, and Pv-k12-1 to confirm the existence of a new race of *P. vignae* f. sp. *adzukicola*; IFO30613 (race 3) was used as a control. Seedlings of each cultivar grown in sterilized vermiculite were removed and soaked for 12 h in inoculum suspensions as described above. The disease severity was assessed 2 weeks after transplanting three to five seedlings into mixed soil in pots of 15 cm diameter using the mean disease severity index (DSI) on a scale of 0–3 (0, no symptoms; 1, a slight or restricted lesion on the hypocotyls; 2, an expanding lesion on the hypocotyls; 3, death). The inoculated plants were arranged in a completely randomized design with three replications. The reactions of the various cultivars are shown in Table 1. Isolate Pv-k11 was pathogenic only to cv. Erimo-shozu and was classified as race 1, whereas isolates Pv-o1, Pv-o2, Pv-o3, Pv-h1, and Pv-k12-1 were virulent to all five cultivars, including cv. Urasa-shimane and cv. Syumari. Consequently, the stem rot disease on cv. Syumari was caused by a new race of *P. vignae* f. sp. *adzukicola*, designated race 4. Differential cultivars for the new race were proposed: cv. Erimo-shozu, cv. Kotobuki-shozu, cv. Noto-shozu, cv. Urasa-shimane, and cv. Syumari.

It is not clear how the new race of *P. vignae* f. sp. *adzukicola* developed within such a short time after the previous survey of the frequency of races in Hokkaido during 1995 and 1996 (Makino et al. 1997). No isolate tested in the report at that time was pathogenic to cv. Urasa-shimane, which was thought to be a parental line resistant to the disease. After crossing Tokei no. 494 (derived from cv. Urasa-shimane) with Tokei no. 486 (from cv. Kuro-shozu, which is resistant to both brown stem rot and Fusarium wilt) in 1991, the F_3 generation was subjected to mass selection for resistant progeny at the Kamikawa Experimental Station (Pippu), where race 4 was discovered in 2000 (N. Kondo, unpublished results). A line with multiple resistance, Toiku no. 140, was developed in 1996 from the progeny and released as cv. Syumari in 2000. When new physiological races of *P. sojae* developed, which is the causal agent of soybean stem and root rot, evidence of outcrossing in the field was shown, and the importance of the consequent reassortment of avirulence genes was predicted (Förster et al. 1994). The new races of *P. vignae* f. sp. *adzukicola*, which is a homothallic species like *P. sojae*, may arise by the same mechanism. This should be considered when breeding adzuki beans resistant to *P. vignae* f. sp. *adzukicola*. Further investigation is required to determine the distribution of the race in commercial fields in adzuki bean-producing regions.

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