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DISEASE NOTE

Occurrence of a new race 2.9 of leaf mold of tomato in Japan

Yuichiro Iida · Yasuya Iwadate · Masaharu Kubota · Fumihiro Terami

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Abstract Leaf mold symptoms were found on tomato varieties carrying the *Cf-9* resistance gene against *Passalora fulva*, the causal agent of leaf mold, in Japan in 2008. Disease symptoms and morphological characteristics of the isolates were similar to those of *P. fulva*. After inoculating a set of tomato differentials with the isolates, all isolates were identified as race 2.9 of *P. fulva*, previously unreported.

Keywords *Passalora fulva* · *Cladosporium fulvum* · *Fulvia fulva* · Leaf mold · Tomato · Race · *Cf* resistance genes

Passalora fulva (Cooke) U. Braun & Crous [syn: *Cladosporium fulvum* Cooke, *Fulvia fulva* (Cooke) Ciferri] is a biotrophic pathogen that causes leaf mold of tomato (*Solanum lycopersicum* L.) (Braun et al. 2003; Crous and Braun 2003). The disease is a primary problem on greenhouse tomatoes and occurs around the world when relative humidity is high. Genes conferring resistance to *P. fulva* (*Cf-2*, *Cf-4, Cf-5*, *Cf-6*, *Cf-9,* and *Cf-11*) have been introduced into cultivated tomato from wild species, and these resistance genes show a typical gene-for-gene relationship (Thomma et al. 2005). However, new races that overcome the resistance genes have rapidly evolved, and isolates that can overcome all resistance genes except *Cf-6* have appeared in Europe since the 1980s (Lindhout et al. 1989).

In Japan, the resistance of cultivars that carry *Cf-2*, *Cf-4, Cf-9,* and *Cf-11* has been overcome by eight races of *P. fulva* (Enya et al. 2009; Kishi 1962; Ozaki and Shirakawa 1996; Satou et al. 2005; Yamada and Abiko 2002). Races that have overcome *Cf-9* resistance first occurred in Gunma, Chiba, and Fukushima prefectures in 2007 and were identified as races 4.9 and 4.9.11 (Enya et al. 2009). In 2008, leaf mold was also found on *Cf-9* tomato cultivars in Iwate prefecture, geographically distant from the area where races 4.9 and 4.9.11 have occurred. The present study was undertaken to determine whether the 2008 outbreak involved the same races or a new race had developed.

Disease symptoms are usually limited to the foliage and first appear on the upper surfaces of leaves as pale green or yellow spots with indefinite margins (Fig. 1a). The lower surfaces of the patches become covered with a velvety, olive-brown fungus growth (Fig. 1b). As the disease progresses, the leaf spots turn yellowish brown. The leaves curl, wither, and drop prematurely. Defoliation starts at the bottom of the plant and progresses upward. In 2008, five single-spore isolates were obtained from cvs. 'Momotaro-Sunny' and 'Momotaro-Natsumi' (Takii & Co., Kyoto, Japan), hybrid cultivars that carry *Cf-9*. Their conidia were catenate, acropleurogenous, pale to dark brown, cylindrical or ellipsoid, straight or mildly curved, had 0 to 2 septa (mostly 0 or 1), and were 9–29 × 4–8 μm in size on potato dextrose agar (Difco, Detroit, MI, USA; Fig. 1c). Disease symptoms and the morphological characteristics of these isolates were similar to those described previously for *P. fulva* (Holiday and Mulder 1976).

Y. Iida (✉) · M. Kubota · F. Terami
National Institute of Vegetable and Tea Science,
Tsu, Mie 514-2392, Japan
e-mail: y_iida@affrc.go.jp

Y. Iwadate
Plant Disease and Entomology Research Section,
Iwate Agricultural Research Center, 20-1 Narita, Kitakami,
Iwate 024-0003, Japan

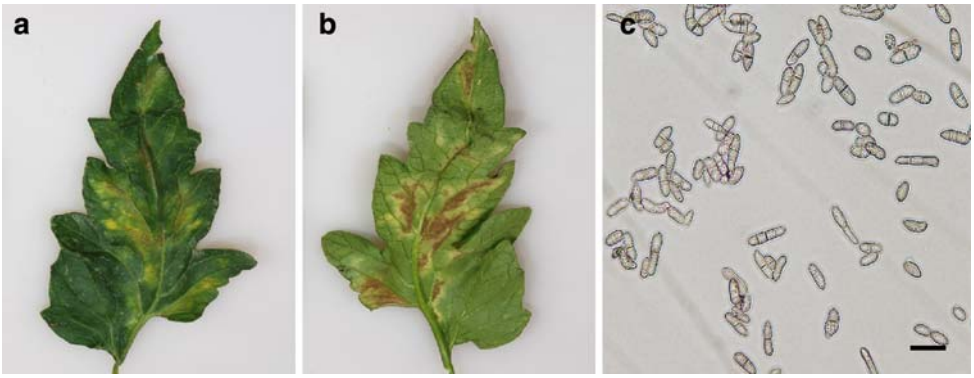

Fig. 1 Symptoms and causal organism of leaf mold of tomato. **a** Typical symptom of a tomato leaf was yellowish spots with indefinite margins on the upper surface of tomato leaf. **b** On the corresponding areas of the lower leaf surface, the fungus appears as an olive green velvety growth with abundant conidia. **c** Conidia produced on potato dextrose agar (PDA). *Bar* 20 μm

To determine the race of the isolates, we tested them on a set of differential genotypes that contained either one or two resistance genes. The tomato cultivars 'Potentate' (no resistance gene), 'Vetomold' (*Cf-2*), 'Purdue 135' (*Cf-4*), 'Moneymaker-Cf-5' (*Cf-5*), 'Ontario 7818' (*Cf-6*), 'Moneymaker-Cf-9' (*Cf-9*), and 'Ontario 7716' (*Cf-4* and *Cf-11*) were used for all inoculations. Isolates were cultured in the dark on potato dextrose agar (Difco) for 2 weeks at 25°C. Three 4-week-old plants of each cultivar were sprayed on the lower side of the leaves with a conidial suspension (10^4 conidia/mL). The inoculated plants were incubated in a moist chamber at 100% relatively humidity and 25°C with a 12 h light/12 h dark photoperiod. After 2 weeks, plants were checked for symptoms and conidia formation. The inoculated plants were scored visually as either resistant or susceptible based on disease development. Inoculation tests were repeated twice for each strain.

All five isolates were virulent on 'Potentate', 'Vetomold', and 'Moneymaker-Cf-9' and generated a large number of conidia on the upper and lower sides of the leaves (Table 1). Other cultivars showed resistance to all isolates by means of a hypersensitive response. On the basis of the differential interaction of the tomato genotypes with the known *Cf* genes, we propose designating these isolates as a new race 2.9 that has not been reported previously. Recent studies showed that new races of *P. fulva* have appeared within several years of the release and prevalence of new tomato varieties based on *Cf* resistance genes in Japan (Enya et al. 2009; Satou et al. 2005; Yamada and Abiko 2002). In Iwate prefecture, the *Cf-9* varieties have been cultivated since 2006, and new race 2.9 was appeared. Our results suggest that new races of *P. fulva* develop rapidly and have overcome the resistance provided by new *Cf* resistance genes within the last several years.

Of the nine races of *P. fulva* in Japan, six have already appeared in Europe (Lindhout et al. 1989), but races 4.9, 4.9.11, and 2.9 are unique to Japan. Race 2.9 appeared in 2008 on *Cf-9* tomato grown in Iwate prefecture, from which race 2 was isolated in 1996 (Ozaki and Shirakawa 1996). It is likely that some isolates of race 2 have broken down the *Cf-9* resistance; these isolates were designated as race 2.9. We are now focusing on four *AVR* genes (*AVR2*, *AVR4*, *AVR4E*, and *AVR9*). The encoded proteins induce a host-specific resistance in tomato that carries the *Cf-2*, *Cf-4*, *Cf-4E*, and *Cf-9* genes, respectively (Thomma et al. 2005). Several mutations in the *AVR* genes that cause a transition from avirulence to virulence (Stergiopoulos et al. 2007). Future experiments will be required to reveal allelic variation in the *AVR* genes of these indigenous races.

Table 1 Virulence spectrum of races of *Passalora fulva* isolated in Japan on tomato genotypes

Tomato genotypes	Resistance genes	Races								
		0	2	2.4	2.4.11	4	4.11	4.9	4.9.11	2.9[a]
Potentate	–	S	S	S	S	S	S	S	S	S
Vetomold	*Cf-2*	R	S	S	S	R	R	R	R	S
Purdue 135	*Cf-4*	R	R	S	S	S	S	S	S	R
Moneymaker-Cf-5	*Cf-5*	R	R	R	R	R	R	R	R	R
Ontario 7818	*Cf-6*	R	R	R	R	R	R	R	R	R
Moneymaker-Cf-9	*Cf-9*	R	R	R	R	R	R	S	S	S
Ontario 7716	*Cf-4*, *Cf-11*	R	R	R	S	R	S	R	S	R

S susceptible, *R* resistant

[a] New race identified in this study

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