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DISEASE NOTE

The first occurrence of leaf mold of tomato caused by races 4.9 and 4.9.11 of *Passalora fulva* (syn. *Fulvia fulva*) in Japan

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Abstract Tomato leaf mold caused by *Passalora fulva* was found on two tomato varieties carrying the *Cf-9* gene in Japan, in 2007. The isolates obtained from Chiba and Fukushima were identified as race 4.9.11, and those from Gunma were races 4.9 or 4.9.11. This is the first report in Japan of tomato leaf mold caused by *P. fulva* strains that can overcome the *Cf-9* gene.

Keywords *Passalora fulva* (*Fulvia fulva*) · Leaf mold · Tomato · Race · *Cf* gene

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Introduction

Tomato (*Solanum lycopersicum* L.) is an economically valuable crop that is mainly cultivated in greenhouses in Japan. The biotrophic fungus *Passalora fulva* (Cooke) Braun and Crous [synonym: *Fulvia fulva* (Cooke) Ciferri] (Crous and Braun 2003) is a causal agent of leaf mold of tomato, which increases rapidly under conditions of high humidity, such as in greenhouses. Single dominant resistance genes have been reported in tomato plants and have been used to develop varieties resistant to leaf mold. In commercial tomato varieties, a number of genes that confer resistance to *P. fulva* have been introgressed from wild tomato species (Thomas et al. 1998). The *Cf-2*, *Cf-9*, and *Cf-11* genes were introgressed from *S. pimpinellifolium* L., *Cf-4* was derived from *S. habrochaites* S. Knapp and D. M. Spooner, and *Cf-5* was derived from *S. lycopersicum* var. *cerasiforme* (Dunal) D. M. Spooner et al. Nevertheless, new races of the pathogen that can overcome the resistance genes have rapidly evolved, and the isolates that can overcome all these resistance genes, including *Cf-5* and *Cf-9*, have appeared in Europe from 1980 s (Lindhout et al. 1989).

Six races of *P. fulva* (0, 2, 4, 2.4, 4.11, and 2.4.11) have been described in Japan (Kishi 1962; Kishi and Abiko 1976; Nii et al. 2005; Ozaki and Shirakawa 1996; Satou et al. 2005; Yamada and Abiko 2002). Although some tomato varieties with the *Cf-9* gene are resistant to all these Japanese races, symptoms of leaf mold were observed on two varieties (cv. Lovely-Ai and Momotaro-Natsumi) with *Cf-9* in Gunma, Chiba, and Fukushima prefectures in 2007. Thus, new races of *P. fulva* seemed to have appeared in Japan. In this report, we investigated the pathogenicity of isolates from these two varieties after inoculating different tomato genotypes that carry known *Cf* gene(s) with the new

isolates, then identified the race composition of these isolates.

Symptoms and morphological characters of the pathogen

Disease symptoms developed on tomato leaves, starting on the lower parts and progressing upward rapidly. The primary leaf symptom was small, white, pale green, or yellowish spots with indefinite margins on the upper surface of the leaves (Fig. 1a). Conidia of the fungal pathogen were olive green to greyish purple and were abundant on the lower surface of the leaves, corresponding to the area of spots on the upper surface (Fig. 1b). The spots became yellowish brown, and the leaves curled, withered, and dropped prematurely. All isolates used in this study produced similar symptoms after inoculation. Conidia of the isolates were catenate, acropleurogenous, pale to dark brown, cylindrical or ellipsoid, straight or mildly curved with 0–2 septa (mostly 0–1), and were 9–35 × 4–8 μm on potato sucrose agar (PSA; 200 g potato, 20 g sucrose, 18 g agar per liter of distilled water; Fig. 1c). Conidiophores of the isolates were pale brown to olivaceous brown, smooth, caespitose, unbranched or occasionally branched, straight or flexuous, narrow at the base, thickening toward the apex, with unilateral nodose swellings that may proliferate as short lateral branchlets (Fig. 1d). Because the disease symptoms and morphological characters of the isolates were similar to those of *P. fulva* described by Holiday and Mulder (1976), the isolates were identified as *P. fulva*.

Fungal isolates and tomato genotypes

We used a total of 22 single-spore isolates of *P. fulva* obtained from two commercial *Cf-9* varieties. Twelve isolates were obtained from cv. Lovely-Ai (Mikado Kyowa Seed Co., Tokyo) in Tomo Area, Gunma prefecture, and five isolates were collected from cv. Lovely-Ai in Kaiso Area, Chiba prefecture. Five isolates were further collected from cv. Momotaro-Natsumi (Takii & Co., Kyoto) in Aizu Area, Fukushima prefecture. Each isolate was maintained at 23°C on potato dextrose agar (PDA; 200 g potato, 20 g dextrose, 18 g agar per liter of distilled water) in a glass tube until use. Tomato genotypes were provided by the C.M. Rick Tomato Genetic Resource Center (TGRC), University of California, Davis CA, USA or the Centre for Genetic Resources (CGN), Wageningen, The Netherlands. Selected genotypes were chosen for the presence of one or two dominant resistance gene(s) against *P. fulva*: 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*; Table 1).

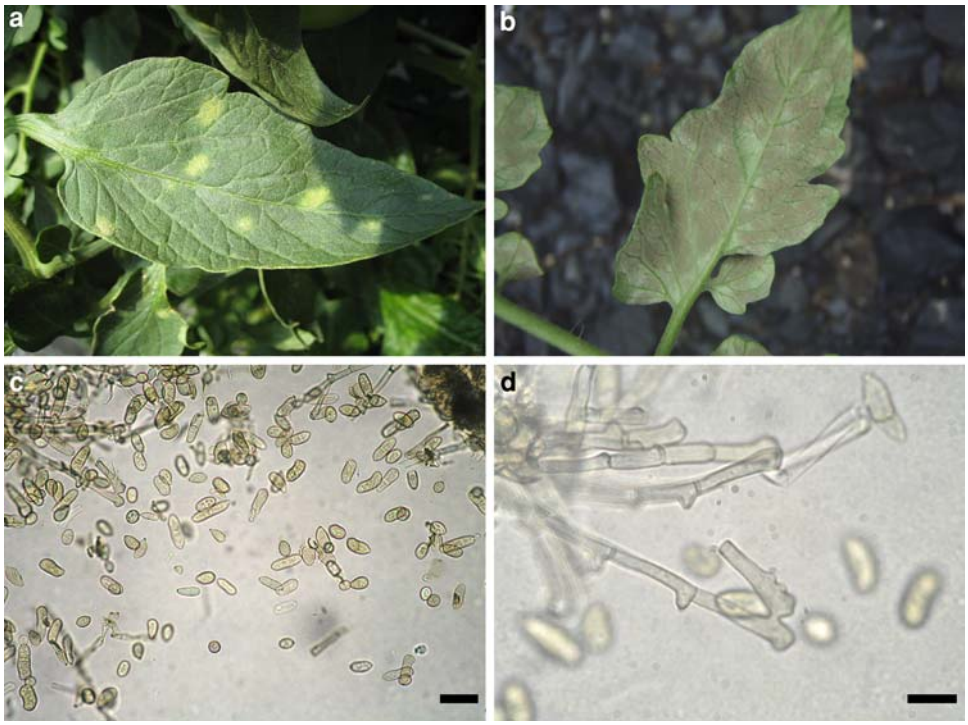

Fig. 1 Disease symptoms and morphological characters of the causal organism of tomato leaf mold. **a** The primary leaf symptom was small and yellowish spots with indefinite margins on the upper surface of the leaf. **b** Conidia formed abundantly on the lower surface of the leaf. **c** Conidia on potato sucrose agar (PSA). *Bar* 20 μm. **d** Conidiophores on PSA. *Bar* 10 μm

Table 1 Reaction of differential sets of tomato varieties against each race of *Passalora fulva*

Tomato genotype	Resistance gene	Races of *Passalora fulva*									
		Reported in Japan						In this study			
								Gunma[a]		Chiba	Fukushima
		0	2	4	2.4	4.11	2.4.11	(*n* = 10) 4.9	(*n* = 2) 4.9.11	(*n* = 5) 4.9.11	(*n* = 5) 4.9.11
Potentate	None	S	S	S	S	S	S	S	S	S	S
Vetomold	*Cf-2*	R	S	R	S	R	S	R	R	R	R
Purdue 135	*Cf-4*	R	R	S	S	S	S	S	S	S	S
Moneymaker-Cf-5	*Cf-5*	R	R	R	R	R	R	R	R	R	R
Ontario 7818	*Cf-6*	R	R	R	R	R	R	R	R	R	R
Moneymaker-Cf-9	*Cf-9*	R	R	R	R	R	R	S	S	S	S
Ontario 7716	*Cf-4*, *Cf-11*	R	R	R	R	S	S	R	S	S	S

S susceptible, *R* resistant

[a] Geographic origin and number of isolates (in parentheses) used in this study

Inoculation and race identification of *P. fulva*

The fungal cultures were streaked on PSA in 9-cm Petri dishes and cultured at 23°C for 14–21 days under dark conditions. Three or four plants of each tomato genotype at the 4- to 5-true-leaf stages were inoculated with *P. fulva* isolates by spraying approximately 30 ml of conidial suspension (10^5 conidia/ml) under and onto the leaflets. The inoculated plants were placed in sweat-boxes to retain 100% relative humidity and then incubated in a growth chamber at 23°C for 13–21 days under a 12-h light/12-h dark cycle. The inoculated plants were scored visually as either resistant or susceptible based on disease development. For each isolate, the experiments were replicated two or three times. The ten fungal isolates obtained from Chiba and Fukushima prefectures were virulent on Potentate, Purdue 135, Moneymaker-Cf-9, and Ontario 7716, and were identified as race 4.9.11 (Table 1). Ten isolates from Gunma prefecture were identified as race 4.9, and two other isolates were identified as race 4.9.11, although they came from the same area (Table 1). This is the first occurrence in Japan of leaf mold of tomato caused by *P. fulva* races that can overcome the *Cf-9* gene.

In a recent study, Yamada and Abiko (2002) investigated race composition of geographically divergent isolates of *P. fulva* obtained during 1997–1998 in Japan, and identified races 0, 2, 2.4, and 2.4.11. Additionally, Satou et al. (2005) found isolates of races 4 and 4.11 in 2003 in Ehime prefecture in western Japan, and Nii et al. (2005) also found races 2.4, 2.4.11, and 4 in 2004 in Fukushima prefecture in northern Japan. These authors suggested that the new races of *P. fulva* appeared within 2 years of release and prevalence of new *Cf*-resistant tomato varieties in areas of Japan. The *Cf-9* varieties have become dominant throughout Japan since 2006 (Sumida et al. 2008). Our results support the suggestion that new races of *P. fulva* have rapidly developed and have overcome the new *Cf* gene in tomato varieties.

In areas infected with races that can overcome *Cf-9*, growers do not use chemicals against leaf mold because chemical control is not recommended for disease control when cultivating a resistant variety. This practice may accelerate the evolution and expansion of new races in a cultivation area because the pathogens can more easily access tomato plants that are subject to less chemical control. This suggests that the control strategy for tomato leaf mold should not only rely on resistant varieties but should be combined with chemical control using effective fungicides. A systematic cultivation protocol that combines resistant tomato varieties with effective chemical and cultural controls should be developed to confront the rapid evolution of the pathogen.

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