New races of the spinach downy mildew pathogen, *Peronospora farinosa* f. sp. *spinaciae*, isolated in Fukuoka Prefecture, Japan in 2019

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Abstract

Five isolates of the pathogen that causes downy mildew in spinach, *Peronospora farinosa* f. sp. *spinaciae*, collected in Fukuoka Prefecture, Japan, in 2019 were identified as two races, which were distinct from races 1–17 authorized by the International Working Group on *Peronospora*. The races were temporarily named J19-1 and J19-2. J19-1 reacted to race-differential cultivars similar to an isolate from the United States, UA201502. However, the response of J19-2 to race-differential cultivars differed from any reported races.

Key words: Spinach downy mildew, *Peronospora farinosa* f. sp. *spinaciae*, novel race

Spinach is a leafy vegetable that is cultivated all year round in Japan. Downy mildew disease of spinach occurs in autumn and spring in warm regions and in summer in cool regions. Because the symptoms affect the leaves, which is the edible part of the plant, the disease causes considerable commercial damage. Although many cultivars with resistance to the disease have been introduced over the years, the disease continues to be a problem in agriculture. New races of the pathogen have appeared one after another and overcome resistance in cultivars.

Many races of the pathogen have been reported. Races 1–17 of the pathogen were characterized by the International Working Group on *Peronospora* (IWGP) based on their reactions to a series of resistant cultivars (Correll et al., 2015; Plantum, 2018). As of 2008, races 3, 4, 5, 6, and 7 had been found in Japan (Satou, 2009; Yamauchi, 2012). Yamauchi et al. (2011) reported the presence of race 8 as well as isolates SAI-1 and SAI-2, which were identified as distinct from races 1–17. Kubota et al. (2017) found races 8, 10, 12, and 13 and a novel race, temporarily referred to as J13-1, that appeared to be distinct from races 1–17, in Japan in 2013–2016. In 2016–2017, three new races were found

in Ibaraki and Saitama Prefectures (Kubota et al., 2019). Two of these, temporarily referred to as J17-2 and J17-3 were novel, and the third, J17-1, appeared to belong to race 17, based on its reactions to a series of cultivars (Plantum, 2018). Races J13-1, J17-2, J17-3 and isolates SAI-1 and SAI-2 differ from each other in their reactions to race-differential cultivars (Kubota et al., 2019).

Seven races, including UA201502, were isolated in the United States in 2013–2017 and did neither correspond to races 1–17 (Feng et al., 2018). An isolate UA4711 from Spain in 2011 was also distinct from races 1–17 (Feng et al., 2014). These isolates were different from Japanese novel isolates, J13-1, J17-2, J17-3, SAI-1 and SAI-2.

In Fukuoka Prefecture, a warmer part in Japan, spinach is actively cultivated in winter and downy mildew disease occurs frequently. We identified races of five isolates of the pathogen occurred in Fukuoka in 2019.

Materials and Methods

We examined isolates of the pathogen recently collected in Fukuoka Prefecture. Five isolates were collected from four towns in 2019 (Table 1). Isolates FA1 and FA2 were collected on February 18 and 21 from the same town, respectively. Isolates F1, F2, and F3 were collected on January 23, February 5, and February 20 from three different towns, respectively. FA1 was isolated from leaves of cultivar cv. Standup 13, which is resistant to races 1–13; and FA2 from cv. Shinbe, which is resistant to races 1–12, 14 and 15. F1 and F2 were isolated from cv. Fukube, which is resistant to races 1–12, 14 and 15. F3 was isolated from cv. Osiris, which is resistant to races 1–10 and 15.

Sporangia of each isolate were collected from diseased leaves and suspended in sterilized water at approximately

Table 1. *Peronospora farinosa* f. sp. *spinaciae* isolates collected in Fukuoka Prefecture in 2019

Isolate	Isolation date	Isolation town	Host cultivar	Host resistance to races
FA1	Feb 18	A	Standup 13	1–13
FA2	Feb 21	A	Shinbe	1–12, 14, 15
F1	Jan 23	B	Fukube	1–12, 14, 15
F2	Feb 5	C	Fukube	1–12, 14, 15
F3	Feb 20	D	Osiris	1–10, 15

10^4 sporangia/ml. The sporangial solutions were sprayed onto spinach plants of the same cultivar from which the isolate was collected, and cv. Hoyo, which has no resistance to the disease. Sporangia produced on the inoculated plants were used in inoculation tests for race identification. Inoculation for race identification was performed as described by Kubota et al. (2017), using a series of 11 cultivars: Viroflay, Resistflay, Califlay, Clermont, Campania, Boeing, Lion, Lazio, Whale, Pigeon and Caladonia. A sporangial suspension adjusted to 10^4 – 10^5 sporangia/ml was sprayed onto leaves and cotyledons placed on 1% agar. Inoculated leaves were incubated at 15°C with a 12 h light/12 h dark cycle for 10 days. Leaves on which sporangia were produced were judged as susceptible. Sporangia produced on leaves after inoculation for race identification were used for repeat inoculations. The inoculation was repeated two to five times for each isolate.

Results and Discussion

Isolates FA2, F1, F2 and F3 were similar to each other in responses to 11 race-differential cultivars (Table 2). These were pathogenic to cvs. Viroflay, Resistflay, Califlay, Clermont, Lazio, Pigeon and Caladonia, and apathogenic to cvs. Campania, Boeing, Lion and Whale. This pattern of reaction to the cultivars did not match the pattern displayed by any of races 1–17 and was therefore temporarily named race J19-1. FA1 infected cvs. Viroflay, Resistflay, Califlay, Clermont, Campania, Boeing, Lion, Whale and Caladonia and did not infect cvs. Lazio and Pigeon. FA1 also did not match the infection patterns of any of races 1–17 and was temporarily named J19-2. Reaction patterns to the cultivars differed among J19-1, J19-2, J17-2 (Kubota et al., 2019), J17-3 (Kubota et al., 2019), J13-1 (Kubota et al., 2017), and SAI-1 or SAI-2 (Yamauchi et al., 2011).

Nine genes involved in resistance to the pathogen have been identified in the race-differential cultivars: *RPF1*–7, 9

and 10 (Feng et al., 2014; 2018) (Table 2). We hypothesized which *RPF* genes each race was able to overcome. J19-1 was able to overcome resistance mediated by *RPF2*, 3, 4, 5 and 9 and J19-2 could overcome that mediated by *RPF1*, 3, 4, 5, 6, and 9; none of the cultivars used in this study possess *RPF7* or 10, so we could not deduce which isolates could overcome resistance mediated by those genes. The pattern shown by J19-1 was similar to that displayed by an isolate collected in the United States in 2015, UA201502 (Feng et al., 2018); note, however, that we did not test for response to *RPF10* which UA201502 could overcome. Patterns of overcoming resistance also differed between J19-1, J19-2, and J13-1 (Kubota et al., 2017). J13-1 was able to overcome resistance mediated by the same genes as race 13. The response to cvs. Califlay and Whale, which both possess *RPF3*, would be affected by emergence of field resistance genes (Correll et al., 2015; Feng et al., 2018). The pattern shown by races J17-2, J17-3, and isolates SAI-1 and SAI-2 could not be defined with respect to *RPF1*–7, 9 and 10, and other undefined resistance genes may also affect the reactions of these isolates on the cultivars.

New mutations affecting gene-for-gene resistance may occur and new races are continuously being identified (Lyon et al., 2016). Oospores of the pathogen have been found on spinach seeds, and we suspect that the new races have been transmitted by seed (Inaba et al., 1983; Kunjeti et al., 2016). However, infection routes of the new races have not been accurately elucidated yet.

Many cultivars possess combinations of resistance genes that are effective against some races of the pathogen; however, mutations that allow the pathogen to overcome each *RPF* are continuously emerging. Although new or previously unknown resistance genes are expected to emerge or be identified, novel races of the pathogen continue to emerge as well. Suppression of the disease by using resistant cultivars is becoming more difficult. Integrated pest management strategies that combine techniques such as chemical use, control of the environment and field sanitation operations, are needed for better suppression of downy mildew of spinach.

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Table 2. Pathogenicity of *Peronospora farinosa* f. sp. *spinaciae* races to spinach cultivars used for race identification

Race or isolate	Spinach cultivars (<i>RPF</i>) ^a											Estimated <i>avr</i> ^b
	Viroflay (none)	Resistflay (5)	Califlay (3)	Clermont (4, 5)	Campania (4, 6)	Boeing (1, 5)	Lion (1, 3)	Lazio (2, 4)	Whale (3)	Pigeon (2, 9)	Caladonia (3, 9)	
FA1 (J19-2) ^c	+	+	+	+	+	+	+	–	+	–	+	1, 3, 4, 5, 6, 9
FA2 (J19-1) ^c	+	+	+	+	–	–	–	+	–	+	+	2, 3, 4, 5, 9
F1 (J19-1) ^c	+	+	+	+	–	–	–	+	–	+	+	2, 3, 4, 5, 9
F2 (J19-1) ^c	+	+	+	+	–	–	–	+	–	+	+	2, 3, 4, 5, 9
F3 (J19-1) ^c	+	+	+	+	–	–	–	+	–	+	+	2, 3, 4, 5, 9
UA201502 ^d	+	+	+	+	–	–	–	+	+	+	+	2, 3, 4, 5, 9
1	+	–	–	–	–	–	–	–	–	–	–	none
2	+	–	+	–	–	–	–	–	–	–	–	3
3	+	+	–	–	–	–	–	–	–	–	–	5
4	+	+	+	–	–	–	–	–	(–)	–	–	3, 5
5	+	+	–	+	–	–	–	–	–	–	–	4, 5
6	+	+	+	+	+	–	–	–	(–)	–	–	3, 4, 5, 6
7	+	+	+	+	–	–	–	–	(–)	–	–	3, 4, 5
8	+	+	–	+	+	+	–	–	–	–	–	1, 4, 5, 6
9	+	+	–	+	+	–	–	–	–	–	–	4, 5, 6
10	+	+	+	+	+	+	+	–	+	–	–	1, 3, 4, 5, 6
11	+	+	–	+	–	–	–	+	–	–	–	2, 4, 5, 6
12	+	+	–	+	+	+	–	+	–	–	–	1, 2, 4, 5, 6
13	+	+	+	+	+/-	–	–	+	+	–	–	2, 3, 4, 5, 6
14	+	+	–	+	+	+	–	+	–	+	–	1, 2, 4, 5, 6, 9
15	+	+	+	–	–	–	–	–	+	–	+	3, 5
16	+	+	–	+	–	–	–	+	–	+	–	2, 4, 5, 9
17	+	+	+	+	+	+	+	+	+	+	+	1, 2, 3, 4, 5, 6, 9
J17-2 ^e	+	–	–	+	–	–	+	+	–	+	–	?
J17-3 ^e	+	–	(–)	+	+	–	–	+	–	+	+	?
J13-1 ^f	+	+	–	+	+	–	–	+	+	N	N	2, 3, 4, 5, 6
SAI-1, 2 ^g	+	–	–	+	+	–	–	–	–	N	N	?

+: susceptible, –: resistant, (–): intermediate resistance, +/-: variable in the numbers of resistant and susceptible plants observed, N: not tested.

Races 1–17 were characterized by the International Working Group on *Peronospora* (Correll et al., 2015; Plantum, 2018).

^a Cultivars' names and code numbers of genes for resistance to *Peronospora farinosa* (*RPF*) (Feng et al., 2014; 2018), ^b Hypothesized codes of avirulence (*avr*) genes corresponding to *RPF*s overcome by each race, ^c Fukuoka isolates and temporary race-names in parenthesis, ^d Feng et al. (2018), ^e Kubota et al. (2019), ^f Kubota et al. (2017), ^g Yamauchi et al. (2011)

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