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Physiological Races and Vegetative Compatibility Groups of *Fusarium oxysporum* f. sp. *lactucae* Isolated from Crisphead Lettuce in Japan

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ABSTRACT

One hundred and sixteen isolates of *Fusarium oxysporum* f. sp. *lactucae* obtained from 85 fields in three crisphead lettuce-producing areas in Nagano Prefecture, Japan were typed for races using differential cultivars Patriot, Banchu Red Fire and Costa Rica No. 4. They were also grouped into vegetative compatibility groups (VCGs) using complementation tests with nitrate non-utilizing (*nit*) mutants. Two California strains reported as *F. oxysporum* f. sp. *lactucum*, a type culture of *F. oxysporum* f. sp. *lactucae*, and 28 avirulent isolates of *F. oxysporum* obtained from crisphead lettuce were included for comparison. Among Nagano isolates, 66 isolates were identified as race 1, and 50 as race 2. Race 1 strains derived from Shiojiri and Komoro cities and race 2 from Kawakami village and Komoro city. All isolates of race 2 were biotin auxotrophs, and the race could be distinguished based on its requirement for biotin on minimal nitrate agar medium (MM). Pathogenic isolates were classified into two VCGs and three heterokaryon self-incompatible isolates. Strong correlations were found between race and VCG. All the race 1 strains were assigned to VCG 1 except self-incompatible isolates, and all the race 2 strains to VCG 2. The 28 avirulent isolates of *F. oxysporum* were incompatible with VCG 1 and VCG 2. California strains was vegetatively compatible with VCG 1, and they were assigned to race 1. Based on vegetative compatibility, these two races of *F. oxysporum* f. sp. *lactucae* may be genetically distinct, and *F. oxysporum* f. sp. *lactucae* race 1 is identical to *F. oxysporum* f. sp. *lactucum*.

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Key words : lettuce root rot, *Fusarium oxysporum* f. sp. *lactucae*, race, vegetative compatibility group.

INTRODUCTION

Fusarium root rot of lettuce (*Lactuca sativa* L.) caused by a vascular wilt pathogen *Fusarium oxysporum* (Schlechtend.: Fr.) f. sp. *lactucae* Matuo *et* Motohashi[17] (FOL) is one of the major factors restricting the stable production of lettuce. The disease was originally reported by Motohashi *et al.* in Tokyo in 1960[18], and a new forma specialis *lactucae* was proposed by Matuo *et al.* in 1967[17]. Matuo *et al.* also reported that FOL was pathogenic both to a butterhead and a crisphead lettuce. Since then, it nearly disappeared for many years. In recent years, however, outbreaks of Fusarium root rot disease on lettuce has been reported in Hokkaido[26], Nagano[3], Shizuoka[16] and Fukuoka[21] Prefectures. In Nagano, the disease was first discoverd in 1995, and has been occurring on crisphead lettuce. On the other hand, except in Nagano, the disease had been occurring on butterhead lettuce. Recently, however, Fusarium root rot of crisphead lettuce has become a serious problem in Nagano, because highly resistant cultivars are not commercially available nor are effective control methods yet available[22].

Fusarium wilt of lettuce was also reported in California, USA, in 1993, and the causal fungus was designated as *F. oxysporum* f. sp. *lactucum* by Hubbard and Geric[7]. They reported that 14 isolates of *F. oxysporum* f. sp. *lactucum* isolated in California were vegetatively compatible with one another. The disease symptoms on lettuce, and the morphological and cultural characteristics of this fungus reported in USA are quite similar to those of Fusarium root rot reported in Japan[4]. The genetic relatedness among the Japanese strains and between the Japanese and Californian strains are not known.

Fujinaga *et al.*[5] first reported the physiological special-

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ization of FOL and proposed two races of FOL isolated from crisphead lettuce, based on three differential cultivar or lines; race 1 is pathogenic to cv. Patriot and line VP1013, and race 2 is pathogenic to cv. Patriot and line VP1010. They also reported[4] that cultivars Costa Rica No. 4 and Banchu Red Fire could be substituted for race-differential lines VP1010 and VP1013; Costa Rica No. 4 and Banchu Red Fire had the same reactions as those of differential lines VP1010 and VP1013, respectively (Table 1). Afterward, Yamauchi *et al.*[30] proposed the pathogenicity groups in FOL on horticultural types of lettuce cultivars. We used the former system of nomenclature because our preliminary study indicated that pathogenicity of FOL did not correspond to horticultural types of lettuce, but to cultivars of lettuce (Fujinaga *et al.*, unpublished data). Furthermore, our system fits the reaction of lettuce cultivars in naturally infested fields in Nagano.

Vegetative compatibility group (VCG) may be used as one method of investigating genetic relatedness among isolates of *F. oxysporum*[13]. In 1985, Puhalla used nitrate non-utilizing (*nit*) mutants of *F. oxysporum* to examine vegetative compatibility[24]. After this work, *nit* mutants have been used to examine VCG, the relationships between races and the VCG, VCG and gene analysis, in many formae speciales. The association between race and VCG has been reported in several pathogens of *F. oxysporum*. Certain populations, such as *F. oxysporum* f. sp. *apii* race 2 and *F. oxysporum* f. sp. *vasinfectum* race 3, were found to be composed of a single VCG[1,10]. In other pathogens, such as *F. oxysporum* f. sp. *melonis* and *F. oxysporum* f. sp. *lycopersici*, the relationship between race and VCG is complex[2,9]. Although Fusarium root rot of lettuce is on the increasing in lettuce-producing areas of Nagano, little is known about the distribution of its races and the relationship between race and VCG.

The objectives of this study are to define the distribution of physiological races of *F. oxysporum* isolates from the lettuce-producing areas of Nagano, to examine the relationship between physiological races and the VCG of FOL, including California strains and the type culture of FOL, and to develop a more rapid race identification method. A preliminary report of this work was already published[23].

Table 1. Race classification of *Fusarium oxysporum* f. sp. *lactucae* according to disease reaction with differential cultivars or lines[a]

Race	Pathogenicity[b]				
	Patriot[c]	Costa Rica No. 4	Banchu Red Fire	VP1010	VP1013
1	+	−	+	−	+
2	+	+	−	+	−

a) According to Fujinaga *et al.*[4,5].
b) +, pathogenic; −, nonpathogenic.
c) Lettuce cvs. Costa Rica No. 4 and Banchu Red Fire and differential cv. or lines Patriot, VP1010 and VP1013.

MATERIALS AND METHODS

Fungal isolates and plant cultivars During 1995 to 2001,144 isolates of *F. oxysporum* from diseased or healthy lettuce plants were collected from three areas (Shiojiri, Komoro, and Kawakami) in Nagano Prefecture, located in central Japan. Each area is more than 60 km apart. Representative strains of two pathogenic variants[5] each from race 1 (SB1-1) and race 2 (F-9501) were included. Most lettuce plants were recovered from fields with a long history of lettuce production. Segments of lettuce stems were surface-sterilized by dipping in 70% ethanol for 30 sec and placed on water agar amended with 0.02% chloramphenicol or on Komada's Fusarium-selective medium[14]. *F. oxysporum* was isolated after 4–7 days of incubation of the plates at 26°C, and single-conidial isolates were made on water agar. A single conidium from these isolates was grown on synthetic low nutrient agar (SNA)[20] or carnation leaf agar (CLA)[19] for morphological determination. Isolates were transferred to potato dextrose agar (PDA) or sterilized compost soil and maintained at 12°C. California strains were received as pure cultures from J.C. Hubbard, U.S. Department of Agriculture, and the type culture of FOL (SUF-762, isolated in Tokyo) was obtained from the culture collection of Shinshu University.

Lettuce cultivars Patriot, Costa Rica No. 4 and Banchu Red Fire were used to determine pathogenicity and race of the collected strains because we previously found that these cultivars were useful in identifying pathogenic variants within FOL[4]. Lines VP1010 and VP1013 were also used in some strains.

Pathogenicity tests and race determination Pathogenicity tests were carried out by directly seeding into infested soil[5]. Inocula were prepared by growing the single-spored isolates grown in wheat bran-vermiculite medium (wheat bran/vermiculite, 1 : 1.5, v/v) for 2 to 3 weeks at 25°C. After incubation, soils were infested by mixing commercial potting soil with one of the inocula (soil/inoculum, 20 : 1, v/v). Seeds of three differential lettuce cultivars were planted in plug trays (200 cells/tray, 2.5×2.5×4.2 cm/cell) filled with the one of the infested soils. Trays were divided into groups infested with only one strain to avoid contamination and incubated in a greenhouse at 22 to 28°C. Similarly, noninoculated seedlings of three differential lettuce cultivars growing in plug trays served as controls. Each isolate was used to

inoculate two replicates of 10 seedlings from each cultivar. External symptoms (leaf necrosis, stunting and plant death) were scored 30 days after seeding. Aerial parts of seedlings were rated for disease on a 0 to 3 scale (0=no symptoms, 1=leaf necrosis, 2=leaf necrosis, stunting and wilt, 3=death). Plants rated at 1.0 and greater than 1.0 were regarded as susceptible and those less than 1.0 as resistant. Isolates were assigned to appropriate races based on their virulence on differential cultivars and were considered avirulent when no symptoms were evident on cultivar Patriot (susceptible to both race 1 and race 2). Standared strains SB1-1 (race 1) and F-9501 (race 2) were included each time to serve as controls to check plant response. Pathogenicity tests were repeated at least once.

Analysis of vegetative growth and auxotrophy Because all strains of race 2 were restricted in growth on Puhalla's minimal nitrate agar medium (MM)[24] and it was difficult to generate *nit* mutants on a chlorate-containing medium (MMC)[24], vegetative growth and auxotrophy of FOL were investigated. Twenty-six strains of race 1 and 38 strains of race 2 were tested for specific nutrition requirements by the method of Holliday[6]. MM was used as a basal medium and were supplemented with different growth factors. Furthermore, mycelial plugs of each strain were plated on MM and MM amended with 500 μg/l of biotin (BMM). Plates were incubated at 25°C for 72 hr, then colony diameters were measured. In sporulation tests, conidia of each strain were counted by the method of Smith *et al.*[27] after cultivation; no distinction was made between micro- and macroconidia.

Vegetative compatibility tests One hundred and sixteen pathogenic isolates, 28 nonpathogenic isolates, two California strains and a type culture (SUF-762) were tested for vegetative compatibility by using complementation of a *nit* mutant. Thirteen isolates of race 1 and 12 isolates of race 2 were initially selected for vegetative compatibility tests. *Nit* mutants were generated from race 1 on MMC and from race 2 on MMC amended with 500 μg/l of biotin (BMMC), respectively. The fast-growing, chlorate-resistant sectors emerged from the initially restricted colonies were transferred to BMM plates. Those that had thin, expansive growth on BMM were considered *nit* mutants. *Nit* mutants were phenotyped according to the methods of Correll *et al.*[1] They were transferred to BMM containing either nitrate, nitrite, hypoxanthine or ammonium as a sole nitrogen source. The plates were incubated at 25°C. Several complementary NitM and *nit1* mutants were selected for each isolate. They were paired in all possible combinations on BMM plates to determine the VCG. When mutants of two different strains formed a heterokaryon, their parents were assigned to the same VCG. After VCGs were established for the initial 25 isolates, two complementary *nit* mutants (NitM) from each VCG that formed robust heterokaryons with many mutants in the same VCG were selected as VCG testers. At least six *nit* mutants from each of the remaining 119 isolates were paired with the VCG testers. Heterokaryon formation was scored on a 1-to-3 scale as described by Kondo *et al.*[15] Those isolates that did not match with any of the VCGs were then tested for heterokaryon self-incompatibility by pairing at least two NitM mutants with 20 *nit1* or *nit3* mutants produced from the same isolate. The tests were repeated at least once.

RESULTS

Race determination

One hundred and forty-four isolates of *F. oxysporum* were isolated from lettuce plants collected from 85 fields of three areas of Nagano (Table 2). One hundred and sixteen isolates were highly virulent on susceptible cv. Patriot, and were identified as FOL (Table 3). Twenty-eight isolates were nonpathogenic, of which 25 were collected from healthy lettuce, and three were from diseased lettuce. FOL could not be isolated from any of the healthy lettuce.

A total of 66 isolates obtained from Shiojiri (31 fields) and Komoro (10 fields) were pathogenic to cvs. Patriot and Banchu Red Fire but not to cv. Costa Rica No. 4. Therefore, they were assigned to race 1. These isolates were mainly obtained from susceptible cultivars Patriot, Shyatoo and Caspar in the fields. All the strains recovered from Shiojiri and 10 of 22 strains from Komoro were race 1. Fifty isolates were pathogenic to cvs. Patriot and Costa Rica No. 4 but not to cv. Banchu Red Fire. Therefore, they were assigned to race 2. Race 2 isolates originated from two areas, Kawakami (38 fields) and Komoro (12 fields) and were mainly obtained from susceptible cultivars Summer Land and Lalaport in the fields. All the strains recovered from Kawakami and 12 of 22 strains recovered from Komoro were race 2.

Vegetative growth and auxotrophy of FOL

All the strains (38 strains) of race 2 had restricted growth on MM (Fig. 1, Table 4), but grew as well as race

Table 2. Locations and numbers of lettuce fields sampled and isolates of *F. oxysporum* found in each location

Location	Fields	No. of isolates
Shiojiri, Nagano Pref.	31	59
Kawakami, Nagano Pref.	32	63
Komoro, Nagano Pref.	22	22
Total	85	144

Table 3. Vegetative compatibility groups, origin, pathogenicity and races of *Fusarium oxysporum* isolated from lettuce collected in Nagano

VCG	Origin	Race[a]	No. of isolates	Source[b]	Virulence[c]
1 subgroup I	Shiojiri	1	48	DP	2.9 (2.2-3.0)
	Komoro	1	10	DP	2.9 (2.8-3.0)
1 subgroup II	Shiojiri	1	5	DP	2.9 (2.7-3.0)
2	Komoro	2	12	DP	2.9 (2.8-3.0)
2	Kawakami	2	38	DP	2.9 (2.5-3.0)
SI[d]	Shiojiri	1	3	DP	2.9 (2.7-3.0)
NC[e]	Shiojiri	—[f]	3	DP	0.0
NC	Kawakami	—	25	HP	0.0

a) Isolate was considered race 1 if it was pathogenic to cv. Banchu Red Fire, and race 2 if it was pathogenic to cv. Costa Rica No. 4.
b) DP, diseased plant; HP, healthy plant.
c) Mean based on disease severity scale on cv. Patriot. Disease severity was assessed with a 0-3 scale. An isolate was considered pathogenic if the mean disease rating was ≧1.0. The numbers in parentheses indicate the range of virulence of isolates in each VCG.
d) SI, Heterokaryon self-incompatible.
e) NC, not vegetatively compatible with isolates in VCGs 1 or 2.
f) —, nonpathogenic.

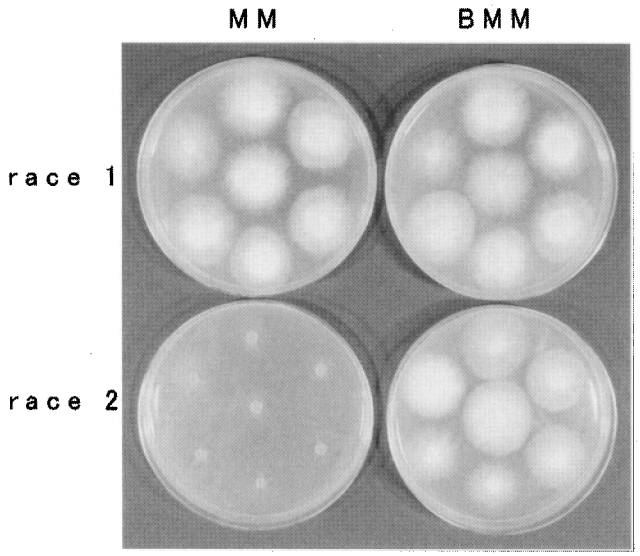

Fig. 1. Growth of the seven race 1 strains (top row) and the seven race 2 strains (bottom row) on MM (right) or MM plus 500 μg biotin/L (BMM, left). Strains were grown at 25°C for 3 days.

1 on PDA. Race 2 strains were identified as biotin auxotrophs by determining their specific nutrition requirements by the method of Holliday. The growth of race 2 on MM was similar to that of wild type by adding biotin (over 1 μg/l). Vegetative growth and sporulation were compared for isolates of race 1 and race 2 of FOL. Growth was evaluated on the basis of colony diameter on MM and BMM. Colony growth of race 2 strains on MM was

Table 4. Vegetative growth of FOL on MM with and without biotin

Race	Origin	Isolates examined	Colony diameters[a]	
			Without biotin	With biotin
1	Nagano	26	37.4±0.39	38.0±1.30
2	Nagano	38	22.1±0.97	35.6±0.51

a) Strains were grown on MM with and without biotin for 72 hr at 25°C, and colony diameters were measured. Each value is the mean (±standard error) of four replications of isolates.

significantly restricted compared with that of race 1 strains. Colonies of race 2 strains were thin or sparse, and aerial hyphae rarely formed, whereas race 1 strains had fluffy growth. On BMM, colony diameter of race 2 strains increased to the same extent as race 1, and aerial hyphae became fluffy (Fig. 1, Table 4).

Seven isolates of race 2 were compared with six isolates of race 1 for sporulation on MM and BMM after 10 days incubation. Race 1 and race 2 strains differed in the number of spores produced on MM (Fig. 2). Race 2 strains produced about 70 times fewer conidia than did race 1. The mean number of spores for race 2 was 3.5×10^4 spores/cm^2 colony, whereas that for race 1 strains was 256.7×10^4 spores/cm^2 colony. On BMM, race 2 strains produced conidia as well as race 1 strains. No significant differences were observed for sporulation of race 1 strains on MM and BMM.

Vegetative compatibility groups

Spontaneous chlorate-resistant sectors were hardly

recovered from any of the race 2 strains of FOL when cultured on MMC, whereas they were readily recovered from all race 1 strains. For race 2 strains, chlorate-resistant sectors were recovered at a mean frequency of 0–0.17 sectors (average 0.06) per colony on MMC, but at 0.3–0.8 sectors (average 0.53) per colony when cultured on BMMC. Chlorate-resistant sectors, when transplanted on BMM, appearing as thin expansive colonies with no aerial mycelium were designated as *nit* mutants.

A group of 25 FOL isolates (13 of race 1, 12 of race 2) from various locations was selected for the initial VCG test. Complementary *nit* mutants (*nit1* or *nit3* and NitM) derived from each isolate were paired in all possible inter-isolate combinations. Based on positive complementation reactions, two VCGs were identified among 25 isolates tested, and two complementary *nit* mutants were chosen as testers of VCG 1 : FL1101-n4 (NitM, parental isolate FL1101, race 1) and FC73-n9 (NitM, parental isolate FC73, race 1). Similarly, complementary NitM tester strains were chosen for VCG 2 : KM-B-n21 (parental isolate KM-B, race 2) and HW-A-n19 (parental isolate HW-A, race 2). However, in the experiments with VCG 1, mutants of five isolates, which formed complementary heterokaryons among themselves, reacted weakly with testers of VCG 1, forming dots of mycelial tufts or growing slowly. Therefore, these five isolates were defined as a subgroup of VCG 1, as described in other formae speciales of *F. oxysporum*[11,12,28]. Then, two additional testers, which formed strong (robust growth was apparent) heterokaryons with the mutants of the five strains, were chosen for this VCG 1 subgroup II: FL341-n12 (NitM, parental isolate FL341, race 1) and FL1110-n6 (NitM, parental isolate FL1110, race 1).

The *nit* mutants were generated from the remaining 116 isolates of FOL and 28 isolates of *F. oxysporum* nonpathogenic to lettuce. The 113 isolates were assigned to either of the two VCGs based on the reaction of their *nit* mutants in pairing with the testers, and no additional VCGs were recognized. Of 113 self-compatible isolates, 58 were assigned to VCG 1 subgroup I (the mutants formed strong heterokaryons with testers of VCG 1), five to VCG 1 subgroup II (the mutants formed strong heterokaryons with FL341-n12 and FL1110-n6), and 50 to VCG 2 (Table 3). No compatibility was found between isolates of different VCGs, and none of the 28 nonpathogenic isolates were compatible with the FOL testers. Three isolates of race 1 were characterized as heterokaryon self-incompatible.

There was a strong correlation between VCG and race (Table 3). All isolates of race 1 were assigned to VCG 1 subgroup I or subgroup II, except heterokaryon self-incompatible isolates. Similarly, all isolates of race 2 were assigned to VCG 2. There were no significant differences

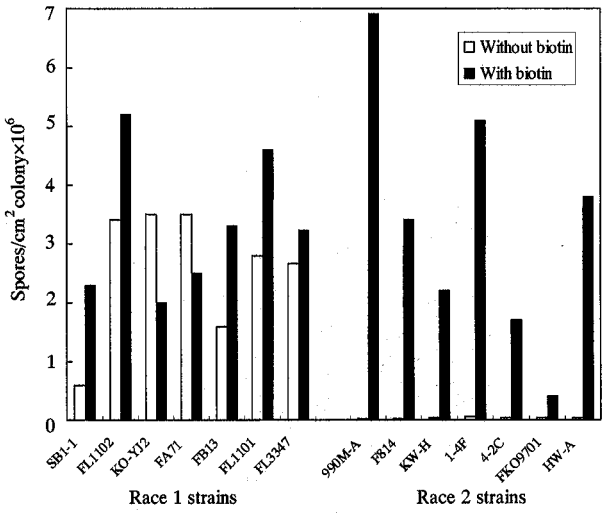

Fig. 2. Comparisons of sporulation of race 1 and race 2 of FOL on MM with and without added biotin. Each value is the mean of three replications.

Table 5. Comparison of VCG and race between Japanese and Californian isolates of *F. oxysporum* f. spp. *lactucae* and *lactucum*

Isolates	Origin	VCG	Race[a)]	Source	Virulence[b)]
SB1-1	Shiojiri, Nagano	1	1	Crisphead	3.0
F-9501	Kawakami, Nagano	2	2	Crisphead	3.0
SUF-762	Tokyo	NC[c)]	NT[d)]	Butterhead	0.3
HL-1	California	1	1	Crisphead	2.8
HL-2	California	1	1	Crisphead	2.2

a) Isolate was considered race 1 if it was pathogenic to cv. Banchu Red Fire, and race 2 if it was pathogenic to cv. Costa Rica No. 4.

b) Mean based on disease severity scale on cv. Patriot. Disease severity was assessed with a 0–3 scale. An isolate was considered pathogenic if the mean disease rating was ≧1.0. The numbers in parentheses indicate the range of virulence of isolates in each VCG.

c) NC, not vegetative compatible with isolates in VCGs 1 or 2.

d) NT, not tested for race identification.

in mean virulence for isolates in VCG 1 and VCG 2.

To compare the VCGs in Nagano with California strains, representative testers of the two local VCGs were paired with complementary *nit* mutants of California strains and the type culture (SUF-762) of FOL (Table 5). Strong complementation was observed in pairing of testers of VCG 1 from Nagano with *nit* mutant of California strains. Thus, California isolates were assigned to VCG 1. The type culture was not vegetatively compatible with the tester isolates of VCG 1 and VCG 2 or with California isolates. It also had much lower disease severity scores.

DISCUSSION

Fusarium root rot of lettuce has been reported in Japan and California, USA[7]. In Japan, the damaged area has been expanding gradually in Nagano, so that this disease is now become one of the most important diseases on crisphead lettuce.

In our preliminary study[5], FOL was revealed to be physiologically specialized, then classified into two races. In this study, FOL isolated from crisphead lettuce grown in Nagano could be race-typed clearly based on its pathogenicity on differential cultivars. The race classification system previously reported[5] has not been routinely used for FOL because the seeds of the race-differential lines VP1010 and VP1013 are not commercially available. But the two cultivars, Banchu Red Fire and Costa Rica No. 4, could substitute for these lines, and race determination should be far less troublesome for researchers if these commercially available seeds are used.

Two physiological races were found among 116 isolates of FOL obtained from crisphead lettuce in Japan. All strains isolated in Shiojiri were race 1, and all in Kawakami were race 2. Both races were isolated in Komoro. In addition, two VCGs were found among FOL. All strains of race 1 were assigned to VCG 1 subgroup I or subgroup II, and all strains of race 2 were assigned to VCG 2. The result clearly revealed a strong correlation between races and VCGs, indicating that these two races of FOL are genetically distinct.

The climate varies in three distinct areas of Nagano with outbreaks of root rot of lettuce. Kawakami is a comparatively temperate area, while Shiojiri is hot, and Komoro varies in climate. Because the cultivar of lettuce should be adapted to climate, the cultivars grown are different for Shiojiri and Kawakami. To put it concretely, Empire[25] type lettuce varieties was used mainly in Shiojiri, and Vanguard[25] type lettuce varieties in Kawakami. The fact that each race was found in Shiojiri and Kawakami may reflect the difference in cultivars grown. That is, the Empire type lettuce varieties has a tendency to be highly susceptibile to race 1, and the Vanguard type lettuce varieties to race 2 (Tsuchiya *et al.*, unpublished data). It would be difficult to determine the origin of FOL in Nagano. But FOL, which was introduced to or endemic in Nagano, apparently was adapted and established in the cultivar of lettuce particular to each area. On the other hand, the cultivars that are common to Shiojiri and Kawakami are grown in Komoro. It is not clear why both races were isolated in Komoro, but it may be due to the use of various cultivars, *i.e.*, the Empire type and Vanguard type lettuce varieties. Hereafter, we need to examine the distribution of the races in Shiojiri and Kawakami in more detail because of the possibility that another race exists in each area.

Hubbard and Geric[7] reported that isolates of *F. oxysporum* f. sp. *lactucum*, causal organism of Fusarium wilt in California, composed a single VCG. But only a small number of isolates was investigated, and may have been insufficient to examine the VCG of *F. oxysporum* f. sp. *lactucum* adequately. In this study, we examined vegetative compatibility among FOL isolates and between FOL and *F. oxysporum* f. sp. *lactucum*. California strains were vegetatively compatible with race 1 of FOL. In addition, they were typed as race 1 based on pathogenicity to differential cultivars, and no morphological differences were observed between California strains and FOL isolated in Japan[4]. Therefore, it is reasonable to think that California strains are identical to race 1 of FOL. As FOL was first reported by Motohashi *et al.*[18], the name of the forma specialis should be unified to avoid confusion. Virulence of the type culture (SUF-762) on susceptible cv. Patriot was weak, and its race could not be determined. It might have lost virulence after the long storage in culture.

Because all strains of race 2 were biotin auxotrophs, it was difficult to generate *nit* mutants on medium without biotin. Yamauchi *et al.*[29] also reported that a difference in biotin requirement was found between 97KO (race 2 of FOL, isolated from crisphead, Nagano) and HS14 (race unknown, butterhead, Shizuoka). Smith *et al.*[27] also reported on biotin auxotrophy of *Fusarium* spp. In our study, biotin auxotrophy was revealed to be a specific trait of race 2 of FOL. Inoue *et al.*[8] reported that one of the pathogenicity-impaired mutants of *F. oxysporum* f. sp. *melonis*, generated using restriction enzyme-mediated integration (REMI) mutagenesis, was biotin auxotrophic. In contrast, race 2 of FOL was highly pathogenic, in spite of its biotin auxotrophy. So, biotin auxotrophy is not likely to be related directly to pathogenicity in the case of race 2 of FOL and is disadvantageous to their survival in nature. Isolating race 2 of FOL from soil may be possible by utilizing its biotin auxotrophy, if biotin auxotrophy is specific in nature to race 2 of FOL, among *F. oxysporum*

isolates including saprobes in the soil of the lettuce fields. Thus, the application of biotin auxotrophy as a marker may be very useful in the study of the ecology of FOL. Studies regarding the ecology of FOL are now under way.

Physiological races of FOL isolated from crisphead lettuce could be distinguished using a vegetative compatibility test as well as a pathogenicity test. The race also could be distinguished based on its requirement for biotin on MM. In our study, two races of FOL seemed to be genetically distinct. We will have to prove the genetic difference between race 1 and race 2.

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