

Assessment of vegetative compatibility of race-2 tomato wilt isolates of *Verticillium dahliae* in Japan

Hideyuki Nagao^{1)*}, Toshimasa Shiraishi²⁾, Satoko Oshima³⁾, Masanori Koike⁴⁾ and Tsutomu Iijima⁵⁾

¹⁾ Faculty of Horticulture, Chiba University, Matsudo 271, Japan

²⁾ Gunma Horticultural Experiment Station, Gunma 379–22, Japan

³⁾ Sakata Seed Co. Ltd., 358, Uchikoshi, Sodegaura, Chiba 299–02, Japan

⁴⁾ Verticillium Research Group, Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080, Japan

⁵⁾ Tokyo Metropolitan Agricultural Experiment Station, Tokyo 180, Japan

Accepted for publication 10 October 1997

Verticillium dahliae race-2 can invade the resistant cultivars of tomato possessing the *Ve* gene. This new race was recently found in several regions in Japan, and 10 isolates of *V. dahliae* race-2 from these regions were used in our study. Pathogenicity tests identified these isolates as the tomato pathotype (B). We examined the vegetative compatibility of 8 of these 10 Japanese isolates of *V. dahliae* race-2 to estimate their genetic relatedness with the testers of Japanese vegetative compatibility group previously proposed (VCGJ) using *nit* mutants. Compatible *nit1* and NitM mutants were obtained from all *V. dahliae* race-2 isolates. Selected representative *nit1* and NitM mutants of each *V. dahliae* race-2 isolates were paired with VCGJ testers. All isolates of *V. dahliae* race-2 showed a strong reaction with VCGJ2, i.e., tomato pathotype. All isolates of *V. dahliae* race-2 except for isolate To22 reacted weakly to VCGJ1 and J3. Japanese isolates of *V. dahliae* race-2 were assigned as VCGJ2 and were hence vegetatively closely related with those of *V. dahliae* race-1. The origin of Japanese isolates of *V. dahliae* race-2 was discussed.

Key Words—*nit*; tomato; VCG; *Ve* gene; *Verticillium dahliae* race-2.

Verticillium dahliae Kleb. invades different kinds of plants including crops (Strausbaugh, 1993; Subbarao et al., 1995) and trees (Chen, 1994; Harada and Murata, 1994; Rijkers et al., 1992), and causes vascular wilt. Resistant cultivars have been developed in tomato by introducing the dominant *Ve* resistant gene (Schaible et al., 1951). An isolate was reported to overcome these resistant cultivars in the USA in 1960 (Alexander, 1962) and designated *V. dahliae* race-2. Subsequently, *V. dahliae* race-2 isolates were reported from Europe (Tjamos, 1981), Australia (O'Brien and Hutton, 1981), and Morocco (Besri et al., 1984). In Japan, the appearance of *V. dahliae* race-2 was reported from Kanagawa Pref. (Kobayashi et al., 1989). Since then, *V. dahliae* race-2 has been reported from other regions.

Verticillium dahliae race-2 isolates can cause severe symptoms in tomato cultivars possessing the *Ve* gene and also are pathogenic to susceptible cultivars (O'Garro and Clarkson, 1988a; Pegg, 1974). Peptide toxin produced by race-2 isolates of *V. dahliae* may be the reason for its pathogenicity toward *Ve* tomato plants (Nachmias et al., 1987).

Cropping systems affect the broadening of host range of *Verticillium* spp. (Tjamos, 1981): monoculture of nonsolanaceous hosts seems to favor strains non-

pathogenic to tomato, and a mixed cropping system, including solanaceous hosts, seems to favor virulent strains. In fact, the first record of race-2 of *V. dahliae* in Greece originated from a diseased eggplant in a mixed cropping system.

The origin of *V. dahliae* race-2 was examined by use of auxotrophic mutants and it was shown that each *V. dahliae* race-2 isolate was complemented only by isolates from the same geographical location (O'Garro and Clarkson, 1988b). O'Garro and Clarkson (1988b) supposed that each *V. dahliae* race-2 isolate coincidentally emerged from the local population of *V. dahliae* race-1. *Verticillium dahliae* was classified into four VC groups using nitrate nonutilizing (*nit*) mutants (Joaquim and Rowe, 1990, 1991). In these studies, isolates of *V. dahliae* were not distinguished by geographical origin. Daayf et al. (1995) reported that race-2 belonged to the different VCGs, VCG2 and VCG4. Japanese isolates of *V. dahliae* formed a VCG including three subgroups (J1, J2, and J3) (Wakatabe et al., 1997). These subgroups were provisionally distinguished according to the strength of heterokaryon. Subgroup J2 comprised isolates which were pathogenic to eggplant and tomato. There was a close relationship between pathogenicity to tomato and subgroup J2.

In this study, we examined vegetative compatibility relationship using *nit* mutants between Japanese isolates

* To whom correspondence should be sent.

of *V. dahliae* race-2 and the testers of Japanese vegetative compatibility groups. A part of this study was previously reported (Nagao et al., 1995).

Materials and Methods

Isolates The origins of isolates of *V. dahliae* race-2 examined in this study are listed in Table 1. Isolates were previously examined for their pathogenicity to the resistant cultivar Okitsu Sango (Shiraishi, personal communication; Oshima, personal communication).

Inoculum production Isolates were cultured on potato-sucrose agar (PSA) for 7–10 d at 25°C. Agar blocks containing mycelia were cut from the margins of colonies and transferred into 500-ml Erlenmeyer flasks containing 50 ml of potato-sucrose broth. Flasks were kept in the dark for 14 d at 25°C. Growing mycelial mats were washed with sterile distilled water and fragmented with chopsticks in 100 ml of sterile distilled water.

Pathogenicity test Pathogenicity tests were conducted using a set of differential host plants (eggplant cv. Senryo-Nigo, sweet pepper cv. Ace, tomato cv. Ponderosa, and Chinese cabbage cv. Yokozuna-Nigo) to determine the pathogenicity groups (Horiuchi et al., 1990). In our study, Chinese cabbage was used as host plant in place of turnip (Horiuchi et al., 1990). The seeds were sown in sterilized soil. After 2–3 wk, seedlings were removed from soil, rinsed in running tap water, and dipped in a mycelial suspension for 10 min. The inoculated seedlings were replanted in plastic nursery containers (40×60 cm) containing sterilized soil. During 6 wk of cultivation, plants were inspected for symptoms every day. Severely wilted plants were occasionally uprooted, and attempts were made to isolate the causal fungi on the acidified water agar medium.

After 6 wk, disease severity was assessed from both external (foliar damage) and internal symptoms, the degree of vascular discoloration of root or hypocotyl. Foliar damage was evaluated by the following scale: 1=no foliar symptoms; 2=yellowing of leaf; 3=wilt or

necrosis of leaf; 4=wilting or necrosis of all leaves; 5=death of all leaves and apical buds. Vascular discoloration was evaluated according to the following scale: 1=no discoloration; 2=browning localized below hypocotyl level; 3=browning of vessels but not of adjacent tissues; 4=browning of both vessels and adjacent tissues. Average rating scales were calculated for each isolate. Horiuchi et al. (1990) proposed four pathogenicity groups, eggplant pathotype (A), tomato pathotype (B), sweet pepper pathotype (C), and crucifera pathotype (D). Pathogenicity group was determined from the results of disease severity.

Recovery and characterization of *nit* mutants *Nit* mutants were generated according to a modified method of Puhalla (1985). Agar blocks (2 mm³) of wild-type colonies of *V. dahliae* growing on PSA were placed on minimal agar medium (MM; according to Puhalla (1985)) containing 3.0% (w/v) potassium chlorate (MMC) in 90-mm Petri dishes. Plates were incubated for 21–28 d at 25°C. Sectors grew from the margin of restricted colonies on this medium. Thin mycelial growth colonies considered to be chlorate-resistant sectors were transferred to both PSA and MM in which the sole nitrogen source was nitrate (2.0 g/L). Colonies growing only on MM as expansive colonies with thin mycelial growth without aerial mycelium after 5 d of incubation were selected as *nit* mutants. Then all *nit* mutant phenotypes were determined by results of growth on all media amended with one of the following nitrogen sources in place of sodium nitrate: sodium nitrite (0.4 g/L), hypoxanthine (0.5 g/L), ammonium-tartrate (0.8 g/L), or uric acid (0.2 g/L) (Correll et al., 1987; Cove, 1976). This test for phenotype was repeated twice. For consistency with previously reported information (Correll et al., 1988; Joaquim and Rowe, 1990), two different phenotypes were selected: *nit1*, unable to utilize nitrate but able to use other nitrogen sources; and *NitM*, unable to use nitrate and hypoxanthine but able to utilize the remaining three nitrogen sources. Another phenotype, *nit2*, was obtained, which was able to utilize solely ammonium.

Complementation tests Pairings were conducted by placing two mycelial blocks of *nit1* and/or *NitM* mutants 15 mm apart on MM in 90-mm Petri dishes. The plates were kept at 25°C for 20 d. Stable complementary heterokaryons were evident by the formation of wild-type growth at the mycelial interface between two *nit* mutants. As previously described (Wakatabe et al., 1997), the most stable *nit1* and *NitM* mutants which gave the strongest reactions were chosen as testers for each isolate. A cellophane barrier was placed between paired *nit* mutants to examine cross-feeding.

Criteria for vegetative compatibility The criteria for vegetative compatibility were evaluated as previously described (Wakatabe et al., 1997). Pairings of *nit1* and *NitM* mutants from each isolate showed wild-type growth greater than 5 mm in width, and this was scored as a positive reaction (++). When an evident line of microsclerotia formed at the mycelial junction of the two mutants but this line developed only slightly (<5 mm), this reaction was considered weak complementation

Table 1. Source of Japanese isolates of *Verticillium dahliae* race-2.

Isolate	Source of isolation (Locality) ^{a)}
To20	Tomato cv. Momotaro (Niiharu, Gunma)
To21	Tomato cv. Healthy (Numata, Gunma)
To22	Tomato cv. Helper M (Shirasawa, Gunma)
To23	Tomato cv. T-92 (Shirasawa, Gunma)
To24	Tomato cv. Momotaro (Shirasawa, Gunma)
To26	Tomato cv. Momotaro (Shirasawa, Gunma)
O101-15	Tomato (Kanagawa)
Vdt102	Tomato (Yamanashi)
Vdt103	Tomato (Yamanashi)
Vdt110	Tomato cv. Sunroad (Misato, Nagano)

a) Isolates were identified and provided by T. Shiraishi (To20, To21, To22, To23, To24, and To26) and S. Oshima (O101-15, Vdt102, Vdt103, and Vdt110).

(+). In other cases, growth was limited to a few small clumps of mycelia and/or microsclerotia along the interface between *nit* mutants. These limited reactions were scored as (-). No reaction between the mutants was scored as (N). Only strongly (++) reacting isolates were used to assign VCGs.

Results

Pathogenicity test Virulence of *V. dahliae* race-2 was estimated on the basis of two criteria: external and internal symptoms (Table 2). All race-2 isolates severely infected eggplant, but disease severity was significantly different between isolates ($P=0.05$). They began to exhibit yellowing of leaves 24 d after inoculation. All race-2 isolates except Vdt103 caused wilting or death of most eggplants in 6 wk. Most race-2 isolates caused only yellowing of leaves on tomato 20 d after inoculation, whereas vascular discoloration extended into the second node. Internal symptoms were found to be significantly different between isolates ($P=0.05$). On the contrary, none of the race-2 isolates affected foliar symptoms on sweet pepper. Browning was observed in a few pepper plants but this was localized at the tip of the primary root. All race-2 isolates except To22 showed a low rating of external symptoms on Chinese cabbage. One isolate of Yamanashi (Vdt103) and five isolates of Gunma (To20, To21, To22, To23, and To24) showed vascular discoloration at the hypocotyl level. Virulence of race-2 isolates on Chinese cabbage was variable and differed significantly between isolates ($P=0.05$).

In summary, the 10 isolates were pathogenic to eggplant and tomato and mildly pathogenic to Chinese cabbage, but not pathogenic to sweet pepper. Pathogenicity tests identified the 10 isolates as the tomato pathotype (B).

Recovery of *nit* mutants Frequencies of chlorate resis-

tant sectors were more than 70.0% in all isolates (Table 3). Ratios of *nit* mutants in totally isolated sectors were not affected by the frequency of chlorate-resistant sectors. Three phenotypically characterized *nit* mutants were obtained, *nit1*, NitM, and *nit2*. Both *nit1* and NitM mutants were obtained for all isolates except To23 and 0101-15. *Nit2* appeared in To20, To26, and 0101-15. All *nit* mutants generated were *nit1* in To23. Frequencies of *nit1* mutant were higher than those of NitM mutants for all isolates except To21.

Vegetative compatibility group Both *nit1* and NitM mutants were generated in 8 of the 10 isolates of *V. dahliae* race-2. We investigated compatibility of these 8 isolates with the testers of Japanese VCGs (Table 4). To20, To21, To22, To24, To26, Vdt102 and Vdt110 were compatible with testers of VCG J2. However, To22, To24 and Vdt102 showed a strong reaction only with one of the testers (LE103). Vdt103 showed a slight reaction with both testers of VCG J2 but no compatibility with other VCGs testers. Only To22 showed strong or slight reaction with the testers of VCG J3. To26, Vdt102, and Vdt103 showed no reaction with VCGJ1 and J3. Compatibility among *V. dahliae* race-2 from the different localities was assessed. Gunma isolate (To20) was compatible with three other Gunma isolates examined (To21, To22, and To24) and with Yamanashi isolate (Vdt103) and Nagano (Vdt110) (Table 5). On the other hand, Yamanashi isolate (Vdt103) and Nagano isolate (Vdt110) showed very weak reaction.

Discussion

Isolates of *V. dahliae* race-2 were pathogenic to eggplant and tomato in inoculation tests and were designated as tomato pathotype (B).

Even though the race-2 isolates examined belonged to the tomato pathotype, their complementary reactions

Table 2. Pathogenicity of Japanese isolates of *Verticillium dahliae* race-2 on a set of differential plants.

Isolate	Disease index ^{a)}											
	Chinese cabbage			Tomato			Pepper			Eggplant		
	n	Ex. index	In. index	n	Ex. index	In. index	n	Ex. index	In. index	n	Ex. index	In. index
To20	9	1.6	2.8	10	2	2.9	10	1	1.1	10	4.9	3.9
To21	9	1.8	2.4	9	2.3	3.1	10	1	1	10	4.7	2.8
To22	9	4	3.1	10	2	2.7	10	1	1	10	4.6	3.2
To23	8	2.3	3	10	2	2.9	10	1	1	10	4.8	3.8
To24	7	2	2.1	9	1.9	2.9	10	1	1.2	10	4.8	3.8
To26	9	1.2	1.6	10	2	2.5	10	1	1.1	10	4.5	3.5
Vdt102	9	1.7	1.6	10	2	3	10	1	1	10	5	4
Vdt103	9	1.8	2.6	9	2	2.8	10	1	1	10	3	3
Vdt110	9	1.8	1.8	10	2.2	2.1	10	1	1	10	4.4	2.9
0101-15	9	1.4	1.7	9	2	3	10	1	1	10	4.8	3.8
F ^{b)}		25.691*	6.875*		1.225	4.465*		0	1.294		19.636*	4.806*

a) Mean values of external symptom (Ex. index) and internal symptom (In. index) were calculated based on replicates (n).

b) Analysis of variance (ANOVA) was performed with $P=0.05$. F values with an asterisk were significantly different.

Table 3. Results of *nit* mutant generation and ratio of *nit* mutant phenotypes of Japanese isolates of *Verticillium dahliae* race-2.

Isolate	No. of colonies inoculated on MMC	No. of generated sectors (%) ^{a)}	No. of <i>nit</i> sectors (%) ^{b)}	Phenotypes of <i>nit</i> (%) ^{c)}			
				<i>Nit1</i>	NitM	<i>nit2</i>	else
To20	20	18 (90.0)	6 (33.3)	50.0	16.7	33.3	0.0
To21	20	23 (115.0)	6 (26.1)	50.0	50.0	0.0	0.0
To22	20	23 (115.0)	11 (47.8)	63.6	36.4	0.0	0.0
To23	32	38 (118.8)	19 (50.0)	100.0	0.0	0.0	0.0
To24	20	16 (80.0)	6 (37.5)	66.7	33.3	0.0	0.0
To26	32	24 (75.0)	13 (54.2)	61.5	23.1	7.7	7.7
O101-15	44	32 (72.7)	18 (54.2)	94.4	0.0	5.6	0.0
Vdt102	16	27 (168.8)	4 (14.8)	50.0	50.0	0.0	0.0
Vdt103	16	22 (137.5)	6 (27.3)	50.0	50.0	0.0	0.0
Vdt110	20	30 (150.0)	16 (53.3)	56.3	18.8	6.3	18.8

a) Number of generated sectors on MMC (percentage = total generated sectors/total inoculated colonies on MMC).

b) Number of *nit* sectors determined (percentage = total determined *nit* sectors/total generated sectors).

c) *Nit3* mutant was not generated.

Table 4. Results of pairings of *nit1* (1) and NitM (M) among Japanese isolates of *Verticillium dahliae*.

VC subgroup		J1		J1		J2		J2		J3		J3	
Mutant		84023		CM208		ST1		LE103		AC406		SM312	
		25	8	11	1	18	28	30	13	14	18	27	30
		(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)
To20	11 (<i>nit1</i>)	N	N	N	N	N	–	N	N	N	–	N	N
	1 (NitM)	–	N	–	N	++	++	++	–	–	N	N	N
To21	5 (<i>nit1</i>)	N	N	–	–	+	++	+	++	N	N	N	N
	8 (NitM)	N	N	–	–	N	++	N	++	N	–	N	N
To22	9 (<i>nit1</i>)	–	N	+	N	N	N	N	+	N	++	N	N
	22 (NitM)	+	N	–	–	+	N	++	N	–	+	+	N
To24	1 (<i>nit1</i>)	N	+	N	+	N	N	N	N	N	N	N	N
	8 (NitM)	N	N	–	–	N	N	++	N	N	–	N	N
To26	14 (<i>nit1</i>)	N	N	N	N	+	–	++	++	N	N	N	N
	16 (NitM)	N	N	N	N	N	++	N	++	N	N	N	N
Vdt102	6 (<i>nit1</i>)	N	N	N	N	N	–	N	++	N	N	N	N
	16 (NitM)	N	N	N	N	+	–	+	N	N	N	N	N
Vdt103	7 (<i>nit1</i>)	N	N	N	N	N	+	N	+	N	N	N	N
	5 (NitM)	N	N	N	N	+	+	–	N	N	N	N	N
Vdt110	1 (<i>nit1</i>)	N	N	N	N	N	–	N	++	N	N	N	N
	25 (NitM)	–	N	–	N	++	++	++	N	–	–	–	N

++, thick wild-type growth and complementation line of more than 5 mm in width; +, slight complementation line of less than 5 mm; –, sparse, limited formation of complemented colonies; N, no reaction.

with VCGJ testers were different from those of race-1 isolates. In our previous report (Wakatabe et al., 1997), VCGJ1 reacted strongly with both VCGJ2 and VCGJ3, but VCGJ2 and VCGJ3 reacted weakly with each other. Subgroups within VCG were proposed based on the weak reaction between the isolates in *Fusarium oxysporum* f. sp. *radicis-lycopersici* Jarvis et Shoemaker (Katan

et al., 1991) and in *V. dahliae* (Strausbaugh et al., 1992). Thus these three groups (VCGJ) were considered to be subgroups in a VCG. In the case of tomato isolates, the VCGJ of race-1 isolates was determined as J2 because race-1 isolates showed strong complementary reaction with the testers of VCG J1 and J2 but slight or limited reactions with those of VCG J3 (Wakatabe et al., 1997).

Table 5. Results of pairings of *nit1* (1) and NitM (M) among isolates of *Verticillium dahliae* race-2.

Mutant		To21		To22		To24		Vdt103		Vdt110	
		5	8	9	22	1	8	7	5	1	25
		(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)
To20	11 (<i>nit1</i>)	++	N	N	+	N	+	N	N	N	++
	1 (NitM)	++	+	++	N	++	+	++	N	++	N
Vdt103	7 (<i>nit1</i>)									N	-
	5 (NitM)									-	N

++, thick wild-type growth and complementation line of more than 5 mm in width; +, slight complementation line of less than 5 mm; -, sparse, limited formation of complemented colonies; N, no reaction; blank, not tested.

Isolates of *V. dahliae* race-2 other than To22 reacted weakly with VCGJ1 and J3. Isolate To22 demonstrated higher disease severity on Chinese cabbage than the race-2 isolates tested (Table 2). However, according to the definition of pathogenicity groups (Horiuchi et al., 1990), pathogenicity to Chinese cabbage does not determine the ascription to the tomato pathotype. The compatibility reaction of To22 was conspicuous among those of race-2 isolates. No race-2 isolate was found to react strongly with only VCG J2 and J3 (Table 4). In our previous report, isolates grouped in VCG J2 showed weak compatibility with VCG J1 and J3 (Wakatabe et al., 1997). This exceptional isolate will be further investigated in vegetative compatibility with the standardized VCGs (Joaquim and Rowe, 1990).

In this experiment, race-2 isolates showed strong reactions with the testers of VCG J2 but almost no complementary reactions with VCG J1 and J3 (Table 4). These results can be interpreted as indicating either that the locality of the isolates is reflected in the complementary reactions, or that isolates of *V. dahliae* race-2 can be categorized as a subpopulation of VCG J2. The former idea was not supported by the results of a previous study (Wakatabe et al., 1997), in which isolates from a local population, Gunma Pref., reacted strongly with the tester isolates of VCG J2. Differentiation in the compatibility reaction existed in the pathogenicity groupings rather than in the locality. In this report, isolates from different regions were paired (Table 5). An isolate (To20) from Gunma Pref. was compatible with race-2 isolates tested in spite of locality, whereas isolates from Yamanashi and Nagano Pref. reacted weakly. Thus the compatibility depended upon the isolates examined rather than the locality. The later idea was supported by the results of vegetative compatibility (Table 4). Unique strong reaction with VCG J2 suggested that isolates of *V. dahliae* race-2 were further genetically isolated from VCG J1 and J3 in terms of vegetative compatibility. We have proposed a VCG including three subgroups for Japanese isolates of *V. dahliae*, in which isolates of *V. dahliae* race-2 could be placed as a subpopulation of subgroup J2. O'Garro and Clarkson (1988b) studied the extent of heterokaryon incompatibility between *V. dahliae* race-1 and race-2 isolates from different geographical origins. They

reported that crossing between North American isolates and Australian isolates failed to produce heterokaryons, but the isolates were fully heterokaryon-compatible within each group, whereas European isolates showed variable compatibility with both the North American and Australian isolates. O'Garro and Clarkson (1988b) pointed out the homogeneity in the North American and Australian isolates and stated that race designation did not appear to affect compatibility. Results of vegetative compatibility among Japanese isolates of *V. dahliae* race-1 and race-2 also suggested the homogeneity of the Japanese isolates. We suppose that Japanese isolates of *V. dahliae* race-2 derived from the Japanese isolates of *V. dahliae* race-1.

Daayf et al. (1995) assessed the relationship of the pathogenicity of *V. dahliae* to cotton and tomato with VCG sensu Joaquim and Rowe (Joaquim and Rowe, 1990). Defoliating strains on cotton belonged to VCG1 and were not virulent on tomato. Non-defoliating strains on cotton belonging to VCGs 2 and 4 were virulent on tomato. A certain degree of specialization among *V. dahliae* strains and the existence of a relationship between pathogenicity and VCGs were supposed (Daayf et al., 1995). However, the relationship between race differentiation and VCGs was complicated. Four isolates of race-1 and an isolate of race-2 of *V. dahliae* belonged to VCG 2 (Daayf et al., 1995). Therefore, in VCG 2, isolates pathogenic to tomato were compatible despite of race differentiation. Daayf et al. (1995) again remarked that another four isolates of race-2 were found in VCG 4, which were self-incompatible and *nit* mutant non-generating. Race-2 isolates thus seemed to be further differentiated in respect to vegetative compatibility.

Some reports have indicated that vegetative compatibility is controlled by the *vic* gene (Elmer, 1991; Puhalla and Spieth, 1985). Therefore, a change in vegetative compatibility due to recombination could occur in the perfect fungi. In fact, sexual recombination resulted in descendants incompatible with the parent isolates in *Gibberella fujikuroi* (Sawada) Ito (Puhalla and Spieth, 1985). In addition to sexual recombinants, Correll et al. (1989) showed the possibility of mutation on the heterokaryon self-incompatibility in *G. fujikuroi*. In perfect fungi, sexual recombinants may result in incompatible descendants

which may further develop as a new vegetative compatibility group. Another possibility of a new vegetative compatibility group may be attributed to heterokaryon self-incompatible isolate. Further favorable mutation in the *vic* gene of this kind isolate may be required to recall the vegetative compatibility. In imperfect fungi, the mechanism of diversity of vegetative compatibility is not known and remains difficult to analyze. However, recent molecular tools have been applied to analyze the genetical diversity of *F. oxysporum*. Isolates of *F. oxysporum* from soil were classified by VCGs and mitochondrial haplotype (mtDNA) (Gordon and Okamoto, 1992a). Some isolates were found to be genetically identical (mtDNA) but vegetatively incompatible (Gordon and Okamoto, 1992b). These isolates were considered to be mutated in a gene which controlled the vegetative compatibility (Appel and Gordon, 1994; Elias et al., 1993). Molecular analysis will be helpful for the assessment of differentiation of vegetative compatibility group.

We have been analyzing Japanese isolates of *Verticillium* spp. using random amplified polymorphic DNA (RAPD) and trying to identify the pathogenicity groups of Japanese *Verticillium* isolates (Koike et al., 1995; Koike et al., 1996). Further study using molecular biology techniques is needed to clarify the relationship between the race differentiation and vegetative compatibility.

Acknowledgement—We gratefully thank Mr. H. Kokubun, Chiba University, for his kind English revision of our manuscript and helpful advice.

Literature cited

- Alexander, L. J. 1962. Susceptibility of certain *Verticillium*-resistant tomato varieties to an Ohio isolate of the pathogen. *Phytopathology* **52**: 998–1000.
- Appel, D. J. and Gordon, T. R. 1994. Local and regional variation in populations of *Fusarium oxysporum* from agricultural field soils. *Phytopathology* **84**: 786–791.
- Besri, M. M., Zroui, M. and Beye, I. 1984. Appartenance raciale et pathogénie comparée de quelques isolats de *Verticillium dahliae* (Kleb.) obtenus à partir de tomates résistantes au Maroc. *Phytopathol. Z.* **109**: 289–294.
- Chen, W. 1994. Vegetative compatibility groups of *Verticillium dahliae* from ornamental woody plants. *Phytopathology* **84**: 214–219.
- Correll, J. C., Gordon, T. R. and McCain, A. H. 1988. Vegetative compatibility and pathogenicity of *Verticillium albo-atrum*. *Phytopathology* **78**: 1017–1021.
- Correll, J. C., Klittich, C. J. R. and Leslie, J. F. 1987. Nitrate non-utilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* **77**: 1640–1646.
- Correll, J. C., Klittich, C. J. R. and Leslie, J. F. 1989. Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). *Mycol. Res.* **93**: 21–27.
- Cove, D. J. 1976. Chlorate toxicity in *Aspergillus nidulans*: The selection and characterization of chlorate-resistant mutants. *Heredity* **36**: 191–203.
- Daayf, F., Nicole, M. and Geiger, J.-P. 1995. Differentiation of *Verticillium dahliae* population on the basis of vegetative compatibility and pathogenicity on cotton. *Eur. J. Plant Pathol.* **101**: 69–79.
- Elias, K. S., Zamir, D., Lichtman-Pleban, T. and Katan, T. 1993. Population structure of *Fusarium oxysporum* f. sp. *lycopersici*: restriction fragment length polymorphisms provide genetic evidence that vegetative compatibility group is an indicator of evolutionary origin. *MPMI* **6**: 565–572.
- Elmer, W. H. 1991. Vegetative compatibility groups of *Fusarium proliferatum* from asparagus and comparisons of virulence, growth rates, and colonization of asparagus residues among groups. *Phytopathology* **81**: 852–857.
- Gordon, T. R. and Okamoto, D. 1992a. Population structure and the relationship between pathogenic and nonpathogenic strains of *Fusarium oxysporum*. *Phytopathology* **82**: 73–77.
- Gordon, T. R. and Okamoto, D. 1992b. Variation within and between populations of *Fusarium oxysporum* based on vegetative compatibility and mitochondrial DNA. *Can. J. Bot.* **70**: 1211–1217.
- Harada, Y. and Murata, K. 1994. *Verticillium* wilt of *Tilia japonica*, a new disease caused by *Verticillium dahliae* Klebahn. *Ann. Phytopath. Soc. Japan* **60**: 757. (Abstr.) (In Japanese.)
- Horiuchi, S., Hagiwara, H. and Takeuchi, S. 1990. Host specificity of isolates of *Verticillium dahliae* towards cruciferous and solanaceous plants. In: *Biological control of soil-borne plant pathogens*, (ed. by Hornby, D.), pp. 285–298. CAB International, Wallingford.
- Joaquim, T. R. and Rowe, R. C. 1990. Reassessment of vegetative compatibility relationships among strains of *Verticillium dahliae* using nitrate-nonutilizing mutants. *Phytopathology* **80**: 1160–1166.
- Joaquim, T. R. and Rowe, R. C. 1991. Vegetative compatibility and virulence of strains of *Verticillium dahliae* from soil and potato plants. *Phytopathology* **81**: 552–558.
- Katan, T., Zamir, D., Sarfatti, M. and Katan, J. 1991. Vegetative compatibility groups and subgroups in *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Phytopathology* **81**: 255–262.
- Kobayashi, M., Komoto, M., Hagiwara, H., Yabuki, S. and Kuniyasu, K. 1989. An isolate of *Verticillium dahliae* invading resistant cultivars of tomato. *Ann. Phytopathol. Soc. Japan* **55**: 500. (Abstr.) (In Japanese.)
- Koike, M., Fujita, M., Nagao, H. and Ohshima, S. 1996. Random amplified polymorphic DNA analysis of Japanese isolates of *Verticillium dahliae* and *V. albo-atrum*. *Plant Dis.* **80**: 1224–1227.
- Koike, M., Watanabe, M., Nagao, H. and Ohshima, S. 1995. Molecular analysis of Japanese isolates of *Verticillium dahliae* and *V. albo-atrum*. *Lett. Appl. Microbiol.* **21**: 75–78.
- Nachmias, A., Buchner, V., Tsrer, L., Burstein, Y. and Keen, N. 1987. Differential phytotoxicity of peptides from culture fluids of *Verticillium dahliae* races 1 and 2 and their relationship to pathogenicity of the fungi on tomato. *Phytopathology* **77**: 506–510.
- Nagao, H., Wakatabe, D., Shiraiishi, T. and Iijima, T. 1995. Vegetative compatibility groups in Japanese isolates of *Verticillium dahliae* using nitrate-nonutilizing mutants. *Phytoparasitica* **23**: 44. (Abstr.)
- O'Brien, R. G. and Hutton, D. G. 1981. Identification of race-2 of *Verticillium* in tomatoes in Southeast Queensland. *Aust. Plant Pathol.* **30**: 56–58.
- O'Garro, L. W. and Clarkson, J. M. 1988a. Pathogenicity of race-1 and race-2 tomato wilt isolates of *Verticillium dahliae* from different geographical origins. *J. Phytopathol.* **123**: 297–303.
- O'Garro, L. W. and Clarkson, J. M. 1988b. Heterokaryon incompatibility and heterozygous diploid production in tomato wilt isolates of *Verticillium dahliae*. *J. Gen. Microbiol.* **134**:

- 2977-2984.
- Pegg, G. F. 1974. *Verticillium* diseases. Rev. Plant Pathol. **53**: 157-182.
- Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. **63**: 179-183.
- Puhalla, J. E. and Spieth, P. T. 1985. A comparison of heterokaryosis and vegetative incompatibility among varieties of *Gibberella fujikuroi* (*F. moniliforme*). Exp. Mycol. **9**: 39-47.
- Rijkers, A. J. M., Hiemstra, J. A. and Bollen, G. J. 1992. Formation of microsclerotia of *Verticillium dahliae* in petioles of infected ash trees. Neth. J. Plant Pathol. **98**: 261-264.
- Schaible, L., Cannon, O. S. and Waddoups, V. 1951. Inheritance of resistance to *Verticillium* wilt in a tomato cross. Phytopathology **41**: 986-990.
- Strausbaugh, C. A. 1993. Assessment of vegetative compatibility and virulence of *Verticillium dahliae* isolates from Idaho potatoes and tester strains. Phytopathology **83**: 1253-1258.
- Strausbaugh, C. A., Schroth, M. N., Weinhold, A. R. and Hancock, J. G. 1992. Assessment of vegetative compatibility of *Verticillium dahliae* tester strains and isolates from California potatoes. Phytopathology **82**: 61-68.
- Subbarao, K. V., Chassot, A., Gordon, T. R., Hubbard, J. C., Bonello, P., Okamoto, D., Davis, R. M. and Koike, S. T. 1995. Genetic relationships and cross pathogenicities of *Verticillium dahliae* isolates from cauliflower and other crops. Phytopathology **85**: 1105-1112.
- Tjamos, E. C. 1981. Virulence of *Verticillium dahliae* and *V. albo-atrum* isolates in tomato seedlings in relation to their host of origin and the applied cropping system. Phytopathology **71**: 98-100.
- Wakatabe, D., Nagao, H., Arai, H., Shiraishi, T., Koike, M. and Iijima, T. 1997. Vegetative combatibility groups of Japanese isolates of *Verticillium dahliae*. Mycoscience **38**: 17-23.