Mycoscience 38: 17–23, 1997

Vegetative compatibility groups of Japanese isolates of *Verticillium dahliae*

Dai Wakatabe[1)*1], Hideyuki Nagao[1)*2], Hiroaki Arai[1)], Toshimasa Shiraishi[2)], Masanori Koike[3)] and Tsutomu Iijima[4)]

[1)] Faculty of Horticulture, Chiba University, Matsudo 271, Japan
[2)] Gunma Horticultural Experiment Station, Gunma 379–22, Japan
[3)] Verticillium Research Group, Laboratory of Forage Crop Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080, Japan
[4)] Tokyo Metropolitan Agricultural Experiment Station, Tokyo 180, Japan

Accepted for publication 19 December 1996

Japanese isolates of *Verticillium dahliae* were examined for vegetative compatibility relationships using nitrate-nonutilizing mutants. Four levels of vegetative compatibility were differentiated according to the degree of compatibility between the tester mutants of *nit1* and NitM. Wild-type growth with a complementation line greater than 5 mm wide was defined as "strong reaction (++)", i.e., compatible. Ten out of 15 isolates showed compatibility and were separated into three groups, provisionally designated as VCGJ1, VCGJ2, and VCGJ3, depending upon their reactions. This method was used to estimate genetic diversity within a local population of *V. dahliae*. Another 12 isolates from Gunma Pref. were paired with tester isolates of the three vegetative compatibility groups proposed. Eight Gunma isolates were assigned to VCGJ1 or VCGJ2. Two isolates were incompatible with all testers. The remaining 2 isolates were self-incompatible. Thus, 18 out of 27 Japanese isolates of *V. dahliae* were assigned to VCGs: 8 to VCGJ1, 7 to VCGJ2, and 3 to VCGJ3. VCGJ1 was compatible with both VCGJ2 and VCGJ3, but VCGJ2 and VCGJ3 showed a weak reaction with each other. Japanese isolates of *V. dahliae* were thus demonstrated to form a VC group comprising three subgroups.

Key Words——genetics; *nit*; pathogenicity group; VCG; *Verticillium dahliae*.

Verticillium dahliae Kleb. is an important vascular wilt pathogen of numerous plant species. Most isolates show a wide host range. Exceptionally isolates of *V. dahliae* from Brussels sprouts and mint proved to be highly host specific (Isaac, 1957; Nelson, 1947). In Japan, Iijima (1983a, b) demonstrated that isolates of *V. dahliae* could be classified into two groups based on their pathogenicity to tomato. Horiuchi *et al.* (1990) classified these isolates into four groups (A, B, C, and D) using four differential host plants (eggplant, sweet pepper, tomato, and turnip). In addition, Suwa et al. (1987) studied the isolates that seriously affected soybean but weakly affected the other differential hosts and proposed the existence of a soybean strain (Es).

Fungal strains that anastomose and form heterokaryons with each other are vegetatively compatible and are assigned to a single vegetative compatibility group (VCG) (Bayman and Cotty, 1991; Brooker et al., 1991; Kuhlman and Bhattacharyya, 1984; Leslie, 1993; Puhalla, 1985). Puhalla (1979) described vegetative compatibility groups among 19 isolates of *V. dahliae* from seven host plants using color mutants, and these groups corresponded partly to virulence on cotton. Puhalla and Hummel (1983) later proposed 16 VCGs for a world-wide collection of *V. dahliae* isolates. In our preliminary study, it was not easy to obtain color mutants from Japanese isolates and recognition of VCGs was unsuccessful (Nagao et al., 1994). Methods using microsclerotial color mutants or auxotrophic mutants had certain inherent disadvantages (Joaquim and Rowe, 1990). When Joaquim and Rowe (1990) used nitrate-nonutilizing (*nit*) mutants to reassess VCGs of *V. dahliae* isolates previously defined by Puhalla and Hummel (1983), number of distinct VCGs was reduced from 16 to 4. A relationship may exist between VCGs and pathogenicity on cotton (Daayf et al., 1995), potato (Joaquim and Rowe, 1991), and tomato (Daayf et al., 1995).

On the contrary, the virulence of *V. dahliae* is exerted as a continuum from weakly virulent to highly virulent strains(Strausbaugh et al., 1992). In terms of anastomosis, occasional formation of a slight aerial mycelium was reported between paired *nit* mutants of different VCGs (Daayf et al., 1995). Studies are still needed, therefore, to recognize the mode of differentiation for incompatibility and its relation to pathogenesis.

In this study, *nit* mutants were used to examine vegetative compatibility relationships among Japanese isolates of *V. dahliae* in order to estimate genetic diversi-

*1 Present address: Nippon Roche Co. Ltd., Kajiwara 200, Kamakura 247, Japan
*2 To whom correspondence should be sent.

ty within and between the different pathogenic strains.

Materials and Methods

Isolates Twenty-seven isolates of *V. dahliae* were used in this study, and their origins are listed in Table 1. All wild-type and *nit* mutants were preserved in the Genetic Resources Center, National Institute of Agrobiological Resources (MAFF), Tsukuba, Japan.

Pathogenicity test All isolates were examined for their pathogenicity to the differential host plants. Isolates were previously cultured on home-made potato-sucrose agar (PSA) for 7–10 d at 25°C. Then agar blocks containing mycelia was cut from the margin of colony and was 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 was fragmented with chopsticks in 100 ml of sterile distilled water.

We investigated the two pathogenicity tests proposed by Iijima (1983b) and Horiuchi et al. (1990). According to Iijima, isolates of *V. dahliae* were distinguished as tomato strain or non-tomato strain by the pathogenicity to susceptible tomato. The susceptible tomato cultivar Beju was used as host in our study. Horiuchi et al. (1990) improved this pathogenicity test for the isolates of non-tomato strain by adding more differential host plants and proposed three additional pathogenicity groups, eggplant strain(A), sweet pepper strain(C), and crucifera strain(D). Four differential host plants (eggplant cv. Senryo Nigo, sweet pepper cv. Ace, tomato cv. Ponte Rosa, and Chinese Cabbage cv. Yokoduna Nigo) were used to determine the pathogenicity groups. In our study, Chinese cabbage was used as host plant in place of turnip (Horiuchi et al., 1990).

Preparation of inocula and cultivation of seedlings

Table 1. Source and pathogenicity of Japanese isolates of *Verticillium dahliae*.

Isolate	Source of isolation and locality	Pathotype	
		By susceptible tomato cultivar[b]	By a set of differential hosts[c]
HE6	Okra, Tokyo	T	B
LE103	Tomato, Tokyo	T	B
LE8602	Tomato, Tokyo	T	B
LE911	Tomato, Nagano	T	B
ST1	Potato, Tokyo	T	B
84011	Chinese cabbage, Aichi[a]	T	B
To-2	Tomato, Gunma	T	B
U-22	Udo (*Aralia cordata*), Gunma	T	B
AC406	Udo (*Aralia cordata*), Gunma	NT	A
84023	Eggplant, Nagano[a]	NT	C
84034	Eggplant, Mie[a]	NT	A
SM312	Eggplant, Tokyo	NT	C
SM821	Eggplant, Tokushima	NT	ND
SM931	Eggplant, Yamagata	NT	A
CM208	Chrysanthemum, Tokyo	NT	A
22720	Chrysanthemum, Shizuoka	NT	C
CV1	Watermelon, Hokkaido	NT	C
C-1	Chinese cabbage, Gunma	NT	C
J-1	Radish, Gunma	NT	A
K-1	Chrysanthemum, Gunma	NT	C
R-1	Rose, Gunma	NT	C
S-1	Strawberry, Gunma	NT	C
So-2	Soybean, Gunma	NT	Es
T-1	Torikabuto (*Aconitum* sp.), Gunma	NT	A
U-48	Udo (*Aralia cordata*), Gunma	NT	C
U-56	Udo (*Aralia cordata*), Gunma	NT	A
Y-1	Gobo-Azami (*Cirsium dipsacolepsis*), Gunma	NT	ND

a) These isolates were kindly supplied by Dr. H. Hagiwara, MAFF, Tsukuba, Japan.

b) T, pathogenic to susceptible cultivar Beju; NT, nonpathogenic to susceptible cultivar Beju.

c) Horiuchi et al. (1990) proposed for pathogenicity groups. A, pathogenic to eggplant and Chinese cabbage; B, pathogenic to tomato, eggplant, and Chinese cabbage; C, pathogenic to sweet pepper, eggplant, and Chinese cabbage; D, pathogenic to Chinese cabbage. Suwa et al. (1987) proposed a soybean strain Es, weakly pathogenic to these differential hosts but uniquely pathogenic to soybean. ND, not determined.

were conducted as below. 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. After 6 wk, plants were scored for vascular discoloration.

Recovery and characterization of *nit* mutants *Nit* mutants can be readily generated using a technique modified from Puhalla (1985). Agar blocks (2 mm^3) of wild-type colonies of *V. dahliae* growing on minimal agar medium (MM, according to Puhalla, 1985) were placed on MM containing 3.0%(w/v) potassium chlorate (MMC) in 8.5-cm 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 $g \cdot L^{-1}$). 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 instead of sodium nitrate: sodium nitrite (0.4 gL^{-1}), hypoxanthine (0.5 gL^{-1}), ammonium tartrate (0.8 gL^{-1}), or uric acid (0.2 gL^{-1}) (Correll et al., 1987; Cove, 1976). This test for phenotype was repeated twice. To be consistent 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 able to utilize solely ammonium.

Complementation tests Pairings were conducted by placing two mycelial blocks of *nit1* and/or NitM mutants 1.5 cm 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

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

Isolate	No. of colonies inoculated on MMC	No. of sectors generated (%)[a]	No. of *nit* sectors (%)[b]	Phenotypes of *nit*(%)[c]			
				nit 1	NitM	*nit 2*	Other
HE6	1100	40 (3.6)	16(40.0)	80.0	20.0	0.0	0.0
LE103	308	55(17.8)	34(61.8)	50.0	50.0	0.0	0.0
LE8602	396	90(22.7)	22(24.4)	36.4	45.4	0.0	18.2
LE911	488	50(10.2)	19(38.0)	23.1	46.2	0.0	30.7
ST1	288	26 (9.1)	15(57.1)	76.0	20.0	0.0	4.0
84011	236	110(46.6)	25(22.7)	60.0	23.3	0.0	16.7
To-2	100	44(44.0)	30(68.2)	68.2	27.3	4.5	0.0
U-22	100	32(32.0)	22(68.8)	68.2	22.7	0.0	9.1
AC406	414	51(12.3)	30(58.7)	94.1	5.9	0.0	0.0
84023	280	101(36.2)	29(28.6)	50.0	31.8	9.1	9.1
84034	232	65(28.0)	51(77.4)	0.0	96.0	0.0	4.0
SM312	600	45 (7.5)	38(84.1)	75.0	25.0	0.0	0.0
SM821	150	18(11.3)	11(64.7)	45.4	36.4	0.0	18.2
SM931	100	36(36.0)	27(75.0)	60.0	20.0	0.0	20.0
CM208	150	39(26.0)	18(46.2)	38.9	27.8	0.0	33.3
22720	472	40 (8.5)	24(60.0)	50.0	25.0	0.0	25.0
CV1	150	50(33.3)	44(88.0)	13.6	86.4	0.0	0.0
C-1	100	28(28.0)	12(42.9)	26.3	47.7	26.3	0.0
J-1	100	44(44.0)	12(27.3)	100.0	0.0	0.0	0.0
K-1	100	29(29.0)	18(62.1)	62.4	18.8	18.8	0.0
R-1	100	12(12.0)	9(75.0)	42.1	57.9	0.0	0.0
S-1	100	50(50.0)	13(26.0)	7.7	84.6	7.7	0.0
So-2	100	24(24.0)	8(33.3)	75.0	25.0	0.0	0.0
T-1	88	37(42.0)	16(43.2)	37.4	43.8	18.8	0.0
U-48	100	24(24.0)	7(26.9)	71.4	0.0	28.6	0.0
U-56	100	36(36.0)	14(38.9)	64.3	28.6	7.1	0.0
Y-1	116	19(16.4)	9(47.4)	11.1	77.8	0.0	11.1

a) Number of sectors generated on MMC (percentage = total generated sectors/total inoculated colonies on MMC × 100).

b) Number of *nit* sectors determined (percentage = total determined *nit* sectors/total generated sectors × 100).

c) *Nit3* mutant was not generated.

growth at the mycelial interface between two *nit* mutants. The strength of a heterokaryon depended on the mutants paired. When the paired *nit1*-NitM mutants were derived from the same parent isolate, strong heterokaryons with wild-type growth were expected. Therefore, the most stable *nit1* and NitM mutants which gave the strongest reactions were chosen as testers for each isolate. To preclude the possibility that paired complementary mutants were not anastomosing but simply cross-feeding extracellularly, a cellophane barrier was placed between paired *nit* mutants.

Criteria for vegetative compatibility The criteria for vegetative compatibility were set by comparison with the wild-type growth between *nit1* and NitM derived from the same parent isolate. All pairings between the representative testers from each isolate showed wild-type growth greater than 5 mm wide, and this was defined as a positive reaction and scored as (++). For a pair of different isolates, the reaction between *nit1* and NitM varied. In some cases, an evident line of microsclerotia formed at the mycelial junction of the two mutants, but this line was slight (<5 mm). This reaction was considered weak complementation and scored as (+). 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.

To determine to which VCGs local isolates (Gunma Prefecture, Japan) of *V. dahliae* belong, *nit1* and NitM of these isolates were paired with the testers determined above.

Results

Pathogenicity test Eight of 27 isolates were pathogenic to tomato and 19 were not. These isolates were further examined by Horiuchi's criteria. Isolates of non-tomato strain were separated into A, C, and Es (Table 1).

Recovery of *nit* mutants Thin mycelial growth colonies on MMC were transferred to both PSA and MM. Colonies which grew as expansive colonies with dense mycelium on PSA but did not grow on MM after 5 d of incubation were considered *nit* mutants. Sectors considered to be *nit* mutants were transferred to media with different nitrogen sources. Frequencies of chlorate-resistant sectors varied depending on the isolates (Table 2). Ratios of *nit* mutants in totally isolated sectors were not affected by the frequency of chlorate-resistant sectors. Three phenotypic *nit* mutants, *nit1*, NitM, and *nit2*, were obtained on various nitrogen sources. Frequencies of each type of *nit* mutant varied depending upon the isolates. Both *nit1* and NitM mutants were obtained from all isolates except 84034, J-1, and U-48, but *nit2* appeared only in some isolates. *Nit3*, unable to utilize nitrate and nitrite but able to utilize the remaining three nitrogen sources, was never generated. In some cases, unidentified *nit* mutants were obtained.

Relationship to vegetative compatibility groups of 15

Table 3. Results of pairings of *nit1* (1) and NitM (M) among Japanese isolates of *Verticillium dahliae*.

Mutant	22720		LE103		LE911		LE8602		ST1		84011		84034	SM312		AC406	
	11(1)	1(M)	30(1)	13(M)	16(1)	9(M)	2(1)	1(M)	18(1)	28(M)	25(1)	15(M)	1(1)	27(1)	30(M)	14(1)	18(M)
84023: 25(1)	−	++	N	++	N	−	N	−	N	+	N	+	N	N	++	N	++
: 8(M)	++[a]	++	++	N	++	−	++	++	++	++	++	N	++	++	++	++	++
22720: 11(1)			N	++	N	++	N	+	N	++	N	++	++	N	++	N	++
: 1(M)			+	++	++	++	++	++	++	+	++	++	++	++	++	++	++
LE103: 30(1)					N	++	N	++	N	++	N	++	−	N	−	N	−
: 13(M)					++	+	++	++	++	++	++	N	+	−	−	+	+
LE911: 16(1)							N	++	N	++	N	++	−	N	N	N	−
: 9(M)							++	++	++	++	++	++	+	−	+	+	+
LE8602: 2(1)									N	++	N	++	+	N	+	N	+
: 1(M)									++	N	++	++	N	+	N	+	+
ST1: 18(1)											N	++	+	N	+	N	+
: 28(M)											++	++	N	−	N	−	+
84011: 25(1)													−	N	−	N	+
: 15(M)													−	−	+	+	+
84034: 1(1)														++	N	++	++
SM312: 27(1)																N	++
: 30(M)																+	++

[a] ++, thick wild-type growth and more than 5 mm wide complementation line; +, thin complementation line less than 5 mm; −, sparsely limited formation of complementation colonies; N, no reaction.

Japanese isolates and their pathogenicity In a preliminary experiment, a pair of *nit1*-NitM mutants from each isolate was selected as representative tester. Growth rates of *nit* mutants varied and sometimes heterokaryon formation was not obvious even with the combination of *nit1*-NitM mutants from the same parent isolate. So *nit* mutants which formed an obvious heterokaryon were chosen as reliable testers. All possible combinations of these tester isolates were tested. Ten of the 15 isolates showed compatible reactions (Table 3). Most pairings showed some degree of heterokaryotic reaction at the margin of the colonies, and these reactions were categorized into three groupings. Both 84023 and 22720 reacted strongly with all isolates and were assigned to group 1. These two isolates were sweet pepper strain. LE103, LE911, LE8602, ST1, and 84011 reacted strongly with each other but all were weakly compatible and/or showed a limited reaction with 84034, SM312, and AC406. They were isolates of tomato strain and were assigned to group 2. 84034, SM312, and AC406 reacted strongly with each other and were assigned to group 3 according to the results of compatibility testing. Isolates in this VCG were 2 isolates of eggplant strain and an isolate of sweet pepper strain. SM931 was self-incompatible and *nit* mutants derived from this isolate were not able to complement one another.

Distribution of VCGs and their strains in a local region Twelve isolates obtained from Gunma Pref. were used. Most isolates were determined as non-tomato strain and originated from different hosts. The relationship between VCGs and the pathogenicity groups was examined in a local region. Frequencies of chlorate-resistant sectors also varied (Table 2). Three phenotypes of *nit* mutants were recognized. More *nit2* mutants were generated from local isolates than the tester isolates. Isolates J-1 and U-48 did not produce NitM.

Ten Gunma isolates were paired with tester strains of the three VCGs determined in the previous test. Six Gunma isolates were assigned to group 1 and two isolates to group 2 (Table 4). However, *nit1* mutants of J-1 and U-48 did not complement with any NitM testers. T-1 and Y-1 were self-incompatible.

Discussion

Nit mutants seem a better tool to detect vegetative com-

Table 4. Results of pairings of *nit1* (1) and NitM (M) among local isolates of *Verticillium dahliae* from Gunma Pref.

Mutant		VCG[a]								
		J1		J2		J2		J3	J3	
		84023		LE103		ST1		84034	AC406	
		25(1)	8(M)	30(1)	13(M)	18(1)	28(M)	1(1)	14(1)	18(M)
C-1	4(1)	N	++[b]	N	++	N	++	++	N	++
	2(M)	+	++	++	N	+	++	N	++	++
K-1	1(1)	N	++	N	+	N	++	++	N	+
	8(M)	++	+	++	++	+	−	++	++	++
R-1	6(1)	N	++	N	+	N	++	++	N	++
	1(M)	++	N	++	++	++	N	++	++	++
S-1	3(1)	N	++	N	+	N	++	++	N	++
	1(M)	++	++	++	+	+	++	++	++	++
So-2	1(1)	N	++	N	+	N	++	++	N	+
	3(M)	++	+	++	++	++	N	++	++	++
U-56	1(1)	N	++	N	+	N	++	++	N	+
	2(M)	++	++	+	−	+	++	++	++	++
To-2	12(1)	N	++	N	++	N	++	+	N	+
	16(M)	+	++	++	++	++	++	+	+	N
U-22	5(1)	N	++	N	++	N	++	+	N	+
	6(M)	−	++	++	N	++	++	N	+	+
J-1	2(1)	N	N	N	N	N	N	N	N	N
U-48	3(1)	N	N	N	N	N	N	N	N	N

a) J1, J2, and J3; three VCGs determined in the previous test.

b) ++, thick wild-type growth and more than 5 mm wide complementation line; +, thin complementation line less than 5 mm; −, sparsely limited formation of complementation colonies; N, no reaction.

patibility than color mutants induced by UV-irradiation. In a previous study using melanin-deficient color mutants, complementary mutants could not be obtained from the isolates of non-tomato strain and compatibility testing was not possible between tomato-strain and non-tomato strain (Nagao et al., 1994).

In this study, we conducted complementation tests with 27 isolates in Japan. Results of the heterokaryotic reaction varied. Chen (1994) reported that the extent of wild-type growth in the complementation tests of *V. dahliae* appeared to depend on the *nit1* mutants and may not necessarily reflect their parent isolates. In our studies, we also found that the strength of a heterokaryon varied depending on the combination of *nit1*-NitM mutants generated from the same parent isolate. So we selected the most stable mutants in each isolate, which showed the strongest reaction when paired. When a positive reaction (++) was used as the criterion for a complementary reaction, 18 out of 27 isolates showed heterokaryon compatibility and were assigned to one of the VCGs depending upon the weak reaction of paired isolates. In this way, 8 isolates (84023, 22720, C-1, K-1, R-1, S-1, So-2, and U-56) were assigned to VCGJ1, 7 isolates (LE103, LE8602, LE911, ST1, 84011, To-2, and U-22) to VCGJ2, and 3 isolates (84034, SM312, and AC406) to VCGJ3. VCGJ1 reacted strongly with both VCGJ2 and VCGJ3, but VCGJ2 and VCGJ3 reacted weakly with each other.

Pathogenicity tests showed that VCGJ2 was composed of isolates virulent to tomato. Isolates of tomato strain also showed vegetative compatibility when examined by melanin synthesis-deficient mutants (Nagao et al., 1994). Compatibility among the isolates of tomato strain was proved again by using different kinds of mutants. Isolates of non-tomato strain were classed into two different VC groups by using *nit* mutants. In terms of the relationship of VCGs with pathogenicity, VCGJ1 and J3 comprised both eggplant strain and sweet pepper strain. An isolate of soybean strain (Es), So-2, was classed into VCGJ1. The relationship of VCGs with pathogenicity is thus not as clear as in the case of tomato strain. However, the reactions of VCGJ1 and VCGJ3 with VCGJ2 (tomato strain) were different. Whereas VCGJ3 and VCGJ2 showed a weak reaction, VCGJ1 strongly reacted with both VCGJ2 and VCGJ3. Most Japanese isolates of *V. dahliae* were compatible with each other even though the degree of compatibility varied among isolates. Thus these three VCGs were considered to be subgroups because of their weak complementary reaction (Fig. 1).

Weak reaction was indicated by the formation of partially heterokaryotic colonies or a sparsely growing hyphal zone in the area of hyphal contact (Chen, 1994). If we assume irreversible evolution in vegetative compatibility, these weak reactions can be interpreted as indicating a distant affinity. VCGJ2 and VCGJ3 are placed on opposite sides of direction in the evolutionary relationship. Assessment of vegetative compatibility of *V. dahliae* using *nit* reduced the number of VCGs, but subgroups were suggested within certain VCGs (Joaquim and Rowe, 1991). Detailed analysis, using more testers and more comprehensive pairings of testers, and also a statistical analysis of the data confirmed the evidence of subgroups within VCG 2 and 4 (Strausbaugh et al., 1992). Katan et al. (1991) reported that VCGs of *F. oxysporum* f. sp. *radicis-lycopersici* have some subgroups. Reaction between subgroups was interpreted as indicating the presence of certain bridging strains. Gordon and Okamoto (1991) discussed the apparent lack of compatibility among isolates of *F. oxysporum* from a field. They detected linkages based on weak reactions between isolates in otherwise distinct VCGs. The existence of subgroups may suggest evolutionary relationships either among strains or among the pathogenic and nonpathogenic strains. In our experiments, the weak reactions suggested the development of a new pathotype or subgroup from VCGJ1. Four VCGs of *V. dahliae* have already been proposed from a study using *nit* mutants (Joaquim and Rowe, 1990). Since we expect VC grouping to be standardized in the future, we provisionally designated the three groups as VCGJ1, VCGJ2, and VCGJ3, respectively.

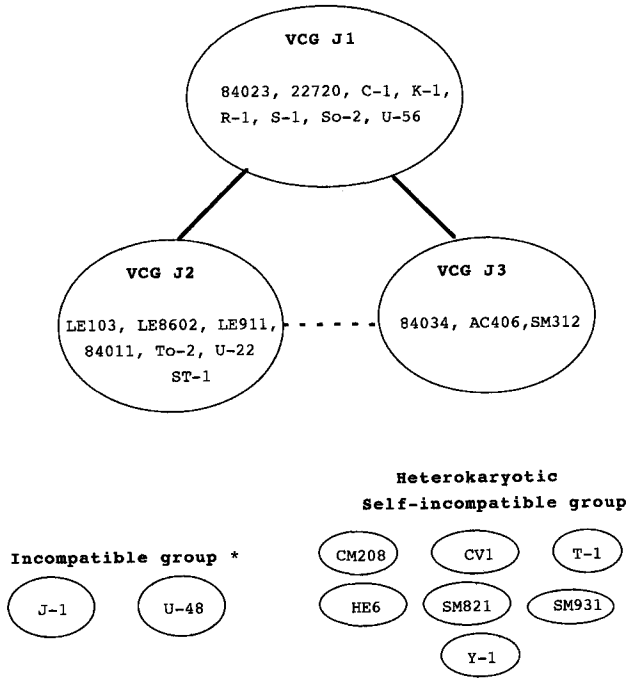

Fig. 1. Interrelationships of Japanese isolates of *Verticllium dahliae* based on vegetative compatibility.
**Nit1* was generated from both isolates but did not react with any of the testers. NitM was not generated.
——, strong reaction; -----, weak reaction.

In *Fusarium*, heterokaryon self-incompatible strains have been identified in field populations (Leslie, 1993). Frequency of recovery from the field was 1–2%, and a widespread geographic distribution is regarded it as a common phenomenon. In our isolates, seven isolates were classed into a self-incompatible group. No relationship was found between strain and self-incompatibility, and the soybean strain did not belong to this group. The

significance of this genetically isolated population remains to be elucidated.

We have thus demonstrated that there was substantial VCG diversity in Japanese isolates of *V. dahliae*, and that vegetative compatibility analysis was a useful tool for classifying isolates of *V. dahliae*.

Acknowledgements——We gratefully thank Dr. A. P. Keinath, Clemson University, USA, for his kind reading of our manuscript and helpful advice and Dr. C. Alabouvette, INRA de Dijon, for revising and correcting our manuscript. This research was supported in part by a Grant-in-Aid for Encouragement of Young Scientists No. 1760042 (1989) from the Ministry of Education, Science and Culture of Japan.

Literature cited

Bayman, P. and Cotty, P. J. 1991. Vegetative compatibility and genetic diversity in the *Aspergillus flavus* population of a single field. Can. J. Bot. **69**: 1707–1711.

Brooker, N. L., Leslie, J. F. and Dickman, M. B. 1991. Nitrate non-utilizing mutants of *Colletotrichum* and their use in studies of vegetative compatibility and genetic relatedness. Phytopathology **81**: 672–677.

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.

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* populations on the basis of vegetative compatibility and pathogenicity on cotton. Euro. J. Plant Pathol. **101**: 69–79.

Gordon, T. R. and Okamoto, D. 1991. Vegetative compatibility groupings in a local population of *Fusarium oxysporum*. Can. J. Bot. **69**: 168–172.

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.

Iijima, T. 1983a. *Verticillium* diseases in Japan. Shokubutu-boeki **37**: 89–95. (In Japanese.)

Iijima, T. 1983b. Studies on *Verticillium* wilt of tomato. Bull. Tokyo Metro. Agric. Exp. Stn. **16**: 63–128. (In Japanese.)

Isaac, I. 1957. *Verticillium* wilt of Brussels sprouts. Ann. Appl. Biol. **45**: 276–283.

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.

Kuhlman, E. G. and Bhattacharyya, H. 1984. Vegetative compatibility and hypovirulence conversion among naturally occurring isolates of *Cryphonectria parasitica*. Phytopathology **74**: 659–664.

Leslie, J. F. 1993. Fungal vegetative compatibility. Ann. Rev. Phytopathol. **31**: 127–150.

Nagao, H., Wakatabe, D. and Iijima, T. 1994. Difficulty to establish vegetative compatibility of Japanese isolates of *Verticillium dahliae* Kleb using melanin synthesis-deficient mutants. J. Gen. Appl. Microbiol. **44**: 277–285.

Nelson, R. 1947. The specific pathogenesis of the *Verticillium* that causes wilt of peppermint. Phytopathology **37**: 17.

Puhalla, J. E. 1979. Classification of isolates of *Verticillium dahliae* based on heterokaryon incompatibility. Phytopathology **69**: 1186–1189.

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 Hummel, M. 1983. Vegetative compatibility groups within *Verticillium dahliae*. Phytopathology **73**: 1305–1308.

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.

Suwa, S., Hagiwara, H., Koumoto, M. and Kuniyasu, K. 1987. Pathogenicity of Soybean *Verticillium* wilt pathogen and its pathogenicity group. Ann. Plant Pathol. Jpn **53**: 379. (Abstr. in Japanese.)