

## How do Japanese isolates of *Verticillium dahliae* correspond with standardized VCG testers?

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We examined the vegetative compatibility of 56 Japanese isolates provisionally assigned to four subgroups of *V. dahliae* to estimate the genetic relatedness with testers of the standardized VCGs. Subgroup J1 was assigned to VCG 2A/B as a new category of assignment. Subgroup J2, except isolate Vdt 110, was assigned to VCG 2A, and subgroup J3, except isolate Vdf 1, was assigned to VCG 2B. Isolates Vdf 1 and Vdt 110 were assigned to VCG 2A/B. Subgroup J4 was assigned to two subgroups, VCG 4B for Vde 1 and VCG 4A/B for FY 3 and HR 1. Four isolates were compatible with both VCG 2 and 4. Isolate U56 was compatible with VCG 2A/B and 4A. Isolates of VCG 2A, Vdt 9 and FF 1, were compatible with either VCG 4A or 4A/B. One isolate of VCG 2B, Vdp-4, was compatible with VCG 4A. Three isolates of subgroup J2 showed weak reactions with the testers of VCG 4. These isolates may be “bridging strains”. Japanese isolates were composed of two VCGs, 2 and 4, “bridging strains” compatible with these VCGs, and some self-incompatible isolates. Testers of VCG 1 and VCG 3 did not show any reactions with the Japanese isolates.

Key Words—bridging strain; pathotype; standardized tester; VCG; *Verticillium dahliae*.

*Verticillium dahliae* Kleb. is one of the important soil-borne fungal plant pathogens. It infects a wide range of crops. As the pathogenicity depends upon the isolate (Bewley, 1922; Kendrick and Middleton, 1959), inoculation tests are required to determine the pathogenicity. In Japan, a differential host method has been proposed (Iijima, 1983) and improved to determine certain host ranges (Hagiwara, 1990; Horiuchi et al., 1990; Suwa et al., 1987). However, this differential host method has not proven conclusive. Over time, as new differential hosts were used for the pathogenicity test, new categorizations resulted (Iijima, 1983; Takeda et al., 1988). Recently, *V. dahliae* race-2 of tomato wilt (Kobayashi et al., 1989) and tomato-pepper pathotype of *V. dahliae* (Iijima, 1983; Oshima et al., 1993) appeared as new diseases in Japan. Now, determination of pathotypes of *V. dahliae* has become quite complicated using the differential host method.

On the other hand, genetic relatedness in fungi can be estimated by their vegetative compatibility (Glass and Kulda, 1992; Leslie, 1993). Joaquim and Rowe (1990) used nitrate-nonutilizing (*nit*) mutants to reassess VCGs (Vegetative Compatibility Groups) of *V. dahliae* isolates

which were previously defined by Puhalla and Hummel (1983). By this method, the number of distinct VCGs was reduced from 16 to 4. Several authors have followed this system (Bao et al., 1998; Daayf et al., 1995; Dobinson et al., 1998; Elena, 1997; Korolev, et al., 1997a, 1997b; Rataj-Guranowska and Hiemsta, 1997; Strausbaugh et al., 1992; Strausbaugh, 1993). It should be emphasized that the method used in VCG analysis can affect the results significantly (Rowe, 1995).

Evaluation of weak reactions led to subdivisions of VCGs 2 and 4. Joaquim and Rowe (1991) discussed differential reactions among isolates of VCG 2 but avoided the subdivision of this VCG in order to keep their VCG system for *V. dahliae* as simple as possible. In the case of VCG 4, three subgroups (4A, 4B, and 4A/B) were proposed to accommodate differential vegetative compatibility reactions of isolates from each subgroup (Joaquim and Rowe, 1991). Strausbaugh et al. (1992) assessed vegetative compatibility by comparing percentages of similarities. In their studies, subgroupings of isolates within VCGs 2 and 4 were also suggested. VCG 2 was subdivided into two subgroups. Subdivisions of VCG 2 were recognized as VCGs 2A and 2B by others (Korolev, et al., 1997a, 1997b). Further subdivision was proposed for VCG 4. Strausbaugh (1993) obtained 33 isolates of *V. dahliae* from 25 potato stems in southern Idaho and proposed nine subgroups of VCG 4.

It was proposed that Japanese isolates of *V. dahliae*

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Table 1. Characteristics of the isolates of *Verticillium dahliae*.

Isolate designation	Origin	Source <sup>a)</sup>	Geographical origin	Pathotype	Standardized VCG	Japanese VCG <sup>b)</sup>	Reference for VCG assignment
<b>Tester strain</b>							
T9	Cotton	1	USA (CA)	Defoliate <sup>c)</sup>	1		Joaquim and Rowe, 1990
V44	Cotton	1	USA (TX)	Defoliate	1		Joaquim and Rowe, 1990
PH	Pistachio	1	USA (CA)	Non-defoliate	2A		Joaquim and Rowe, 1990
WM	Cotton	1	USA (TX)	Non-defoliate	2A		Joaquim and Rowe, 1990
115	Cotton	1	Syria	Non-defoliate	2B		Joaquim and Rowe, 1990
S92	Soil	1	USA (OH)	ND	2B		Joaquim and Rowe, 1990
70-21	Potato	1	USA (OH)	ND	3		Joaquim and Rowe, 1991
PCW	Pepper	1	USA (CA)	ND	3		Joaquim and Rowe, 1990
BB	Potato	1	USA (ID)	Non-defoliate	4A		Joaquim and Rowe, 1990
S39	Soil	1	USA (OH)	Non-defoliate	4B		Joaquim and Rowe, 1990
CW	Cherry	1	USA (WA)	ND	4A/B		Joaquim and Rowe, 1990
<b>Japanese isolates</b>							
CM208	Chrysanthemum	2	Tokyo	A <sup>d)</sup>		J1	Wakatabe et al., 1997
84023	Eggplant	3	Nagano	C		J1	Wakatabe et al., 1997
C-1	Chinese cabbage	4	Gunma	C		J1	Wakatabe et al., 1997
K-1	Chrysanthemum	4	Gunma	C		J1	Wakatabe et al., 1997
R-1	Rose	4	Gunma	C		J1	Wakatabe et al., 1997
S-1	Strawberry	4	Gunma	C		J1	Wakatabe et al., 1997
So-2	Green soybean	4	Gunma	Es		J1	Wakatabe et al., 1997
U56	Udo ( <i>Aralia cordata</i> )	4	Gunma	A		J1	Wakatabe et al., 1997
Vdp-1	Pepper	6	Nagano	C		J1	Nagao et al., 1998
ST1	Potato	2	Tokyo	B		J2	Wakatabe et al., 1997
LE103	Tomato	2	Tokyo	B		J2	Wakatabe et al., 1997
U108	Udo ( <i>Aralia cordata</i> )	4	Gunma	E		J2	Ebihara et al., 1999
To20 <sup>*e)</sup>	Tomato	4	Gunma	B		J2	Nagao et al., 1997
To21 <sup>*</sup>	Tomato	4	Gunma	B		J2	Nagao et al., 1997
To22 <sup>*</sup>	Tomato	4	Gunma	B		J2	Nagao et al., 1997
To24 <sup>*</sup>	Tomato	4	Gunma	B		J2	Nagao et al., 1997
To26 <sup>*</sup>	Tomato	4	Gunma	B		J2	Nagao et al., 1997
Vdb6	Tomato	6	Nagano	B		J2	Nagao et al., 1998
Vdt6	Tomato	6	Nagano	B		J2	Nagao et al., 1998
Vdt7	Tomato	6	Nagano	B		J2	Nagao et al., 1998
Vdt9	Tomato	6	Nagano	B		J2	Nagao et al., 1998
Vdt12	Tomato	6	Nagano	B		J2	Nagao et al., 1998
Vdt102 <sup>*</sup>	Tomato	6	Nagano	B		J2	Nagao et al., 1997
Vdt103 <sup>*</sup>	Tomato	6	Nagano	B		J2	Nagao et al., 1997
Vdt110 <sup>*</sup>	Tomato	6	Nagano	B		J2	Nagao et al., 1997
SM312	Eggplant	2	Tokyo	C		J3	Wakatabe et al., 1997
AC406	Udo ( <i>Aralia cordata</i> )	4	Gunma	A		J3	Wakatabe et al., 1997
So1	Green soybean	4	Gunma	Es		J3	Ebihara et al., 1999
So22	Green soybean	4	Gunma	Es		J3	Ebihara et al., 1999
So23	Green soybean	4	Gunma	Es		J3	Ebihara et al., 1999
So27	Green soybean	4	Gunma	Es		J3	Ebihara et al., 1999
So28	Green soybean	4	Gunma	Es		J3	Ebihara et al., 1999
So39	Green soybean	4	Gunma	Es		J3	Ebihara et al., 1999
So40	Green soybean	4	Gunma	Es		J3	Ebihara et al., 1999
So41	Green soybean	4	Gunma	Es		J3	Ebihara et al., 1999
U54	Udo ( <i>Aralia cordata</i> )	4	Gunma	E		J3	Ebihara et al., 1999
U68	Udo ( <i>Aralia cordata</i> )	4	Gunma	E		J3	Ebihara et al., 1999
U69	Udo ( <i>Aralia cordata</i> )	4	Gunma	E		J3	Ebihara et al., 1999
U90	Udo ( <i>Aralia cordata</i> )	4	Gunma	E		J3	Ebihara et al., 1999
U95	Udo ( <i>Aralia cordata</i> )	4	Gunma	E		J3	Ebihara et al., 1999
U115	Udo ( <i>Aralia cordata</i> )	4	Gunma	Es		32	Ebihara et al., 1999
Vdp-2	Pepper	6	Nagano	C		J3	Nagao et al., 1998
Vdp-4	Pepper	6	Nagano	B+C		J3	Nagao et al., 1998
HR1	Horse radish	7	Hokkaido	E		ND	Ebihara et al., 1999
22201	Eggplant	2	Tokyo	E		ND	
FF1 <sup>*</sup>	Tomato cv. momotaro 8	5	Fukushima	B		ND	
FN1 <sup>*</sup>	Tomato cv. T92	5	Fukushima	B		ND	
FN2 <sup>*</sup>	Tomato cv. momotaro	5	Fukushima	B		ND	
FT1 <sup>*</sup>	Tomato cv. momotaro 8	5	Fukushima	B		ND	
FY2 <sup>*</sup>	Tomato cv. T92	5	Fukushima	B		ND	
FY3 <sup>*</sup>	Tomato cv. momotaro	5	Fukushima	B		ND	
Vdc4	Melon	6	Nagano	ND		ND	
Vde1	Eggplant	6	Osaka	ND		ND	
Vde4	Eggplant	6	Nagano	ND		ND	
Vdf1	Japanese butterbur	6	Osaka	ND		ND	
Vdu1	Udo ( <i>Aralia cordata</i> )	6	Osaka	ND		ND	

a) 1=R.C. Rowe; 2=T. Iijima; 3=H. Hagiwara; 4=T. Shiraiishi; 5=K. Tairako; 6=S. Oshima; 7=M. Koike.

b) Japanese VCG was composed of a VCG including these subgroups (J1, J2 and J3) (Wakatabe et al., 1997). Isolate HR 1 was assigned to another VCG (Ebihara et al., 1999).

c) Defoliate and non-defoliate pathotype for cotton (Daayf et al., 1995; Puhalla, 1979). ND, not determined.

d) A, eggplant pathotype; B, tomato pathotype; C, pepper pathotype; (Horiuchi et al., 1990); E, weakly pathogenic to these differential hosts (Hagiwara, 1990); Es, 'soybean strain' (Suwa et al., 1987); B+C, tomato-pepper pathotype (Oshima et al., 1993). ND, not determined.

e) Isolate with an asterisk is race 2 of tomato wilt.

be grouped as a single VCG with three subgroups using *nit* mutants (Wakatabe et al., 1997). Race 2 of tomato wilt (Nagao et al., 1997), tomato-pepper pathotype (Nagao et al., 1998), and pathotype E within 'soybean pathotype' (Ebihara et al., 1999) were assigned to these three subgroups, except for heterokaryon self-incompatible isolates and HR 1. HR 1 was thought to belong to a different VCG than the Japanese isolates (Ebihara et al., 1999).

In this study, we examined vegetative compatibility among 56 Japanese isolates of *V. dahliae* using testers of the standardized vegetative compatibility groups proposed by Joaquim and Rowe (1990, 1991) and assigned Japanese isolates to standardized VCGs.

### Materials and Methods

**Isolates** The origins of isolates of *V. dahliae* examined in this study are listed in Table 1. Isolates were cultured on potato-sucrose agar at 25°C in darkness. Testers of standardized VCGs were provided by Prof. R. C. Rowe, Ohio University (Import permit No. 6Y2146).

**Recovery and characterization of *nit* mutants for additional isolates** Twelve isolates were newly examined to generate *nit* mutants in this study. *Nit* mutants were recovered according to a modification of the method of Puhalla (1985). Plates were incubated for 21–28 d at 23°C. Vegetative compatibility was evaluated as previously described (Wakatabe et al., 1997). Phenotype was determined by the results of growth on media modified 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 (0.1 g/L of uric acid was used instead of 0.2 g/L) (Correll et al., 1987; Cove, 1976). This test for phenotype was repeated twice.

**Complementation tests** Pairings were conducted with

*nit1* and NitM combination and these tests were repeated twice with two pairs. Complementation was evaluated as previously described (Wakatabe et al., 1997). The plates were kept at 25°C for 20 d.

### Results

**Recovery of *nit* mutants** Frequencies of chlorate-resistant sectors were highly variable and depended on the isolates examined (Table 2). These frequencies did not affect the ratios of *nit* mutants generated in total isolate sectors. These features of *nit* mutants were consistent with those observed in previous studies of *V. dahliae* (Ebihara et al., 1999; Nagao et al., 1997, 1998; Wakatabe et al., 1997). However, *nit 3* mutants were generated in five isolates in this study. Three of these isolates were race 2 of tomato wilt. Another three isolates of race 2 did not generate *nit 3* (Table 2). Isolates which generated *nit 3* concomitantly generated unidentified *nit* mutants. The number of colonies tested did not affect the generation of *nit 3* or unidentified *nit* mutants.

**Vegetative compatibility of 12 additional Japanese isolates** Both *nit1* and NitM mutants were recovered from all 12 Japanese isolates tested. A pair of *nit 1*-NitM mutants was selected as representative testers as previously described (Wakatabe et al., 1997). Ten out of the 12 isolates tested showed compatible reactions when paired with all possible combinations of testers for each of three subgroups of Japanese isolates (J1, J2, and J3). Isolates Vde4, FF1, FN1, FN2, FT1, and FY2 showed strong reactions with the testers of J1 and J2 (Table 3). These isolates were assigned to subgroup J2. Most of these isolates were identified as race-2, except Vde4. These 6 isolates mostly showed compatible reaction among themselves (Table 4). Four miscellaneous isolates (22201, Vdc4, Vdf1, and Vdu1) were compatible with the testers of J1 and J3 (Table 3). These isolates

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

Isolate	No. of colonies inoculated on MMC	No. of generated sectors	No. of <i>nit</i> sectors (%)	Phenotypes of <i>nit</i> (%)				
				NitM	<i>nit 1</i>	<i>nit 2</i>	<i>nit 3</i>	other
22201	32	22	14(63.6)	28.6	71.4	0	0	0
FF1	20	80	30(37.5)	30	70	0	0	0
FN1	20	114	37(32.5)	45.9	51.4	2.7	0	0
FN2	20	108	37(34.3)	21.6	73	5.4	0	0
FT1	20	126	54(42.9)	22.2	63	1.9	7.4	5.6
FY2	20	127	57(44.9)	31.6	50.9	12.3	1.8	3.5
FY3	20	112	60(53.6)	23.3	66.7	1.7	5	3.3
Vdc4	20	70	61(87.1)	88.5	4.9	0	1.6	4.9
Vde1	20	105	44(41.9)	18.2	50	0	18.2	13.6
Vde4	32	59	18(30.5)	33.3	27.8	11.1	0	27.8
Vdf1	32	14	3(21.4)	33.3	66.7	0	0	0
Vdu1	32	23	7(30.4)	57.1	28.6	0	0	14.3

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

were assigned to subgroup J3. These 4 isolates showed compatibility among themselves but not with other isolates (Table 4). Isolates FY3, HR1, and Vde1 did not show strong compatibility with testers of any of the three subgroups (Table 3) (Ebihara et al., 1999). However, these isolates did show limited compatibility with each other and with some other isolates: Vde4, FF1, and FT1 (Table 4). These less compatible isolates were provisionally assigned to a new subgroup, J4. The origins of these isolates were diverse (Table 1).

**Assessment of vegetative compatibility of Japanese isolates with standardized VCG testers.** Eleven tester strains of the VCGs *sensu* Joaquim and Rowe (1990, 1991) were paired with Japanese isolates. Pairings with the testers of VCGs 2 and 4 led to highly variable reactions.

Isolates of subgroup J1 showed compatible reaction with testers of VCG 2B, except Vdp-1 (Table 5). For

testers of VCG 2A, half of these isolates showed compatible reaction with at least one of the testers, but U56 and Vdp-1 did not. As these isolates were highly compatible with other members of this subgroup (Wakatabe et al., 1997; Nagao et al., 1998), all isolates were assigned to the same VCG. Subgroup J1 was assigned to a newly proposed group, VCG 2A/B. Tester PH was not clear enough to demonstrate compatibility for these isolates. Isolate U56 showed compatible reaction with the tester of VCG 4A, weak reaction with the tester of 4A/B, and no reaction with the tester of VCG 4B.

Isolates of subgroup J2 almost all showed strong reactions with the testers of VCG 2A, except ST1, LE103, FY2, To20, To21, Vdt103, and Vdt110 (Table 5). However, tester WM showed sparse and limited heterokayon formation or no reaction with isolates of subgroup J2, although tester WM has been assigned to the same VCG as another tester, PH. Most isolates of

Table 3. Results of pairings with testers of subgroups of Japanese VCG of *Verticillium dahliae*.

VC subgroup		J1		J1		J2		J2		J3		J3	
		CM208		84023		ST1		LE103		AC406		SM312	
		11 (1)	1 (M)	25 (1)	8 (M)	18 (1)	28 (M)	30 (1)	13 (M)	14 (1)	18 (M)	27 (1)	30 (M)
FN1	44[nit1]		–		–		++		++		ND		N
	33[NitM]	++		++		++		++		ND			N
FN2	94[nit1]		–		–		++		++		ND		N
	79[NitM]	++		++		++		++		ND			N
FT1	17[nit1]		–		N		++		++		ND		N
	5[NitM]	–		N		N		N		ND			N
FF1	32[nit1]		++		–		++		++		ND		N
	42[NitM]	+		+		–		–		ND			N
FY2	109[nit1]		–		N		–		++		ND		N
	115[NitM]	–		N		N		–		ND			N
Vde4	42[nit1]		–		–		–		+		N		N
	9[NitM]	–		+		++		++		N			N
22201	7[nit1]		++		++		N		–		++		N
	19[NitM]	++		++		N		N		++			N
Vdc4	27[nit1]		++		++		N		N		++		–
	23[NitM]	++		++		N		N		++			N
Vdf1	14[nit1]		++		++		N		+		++		++
	4[NitM]	++		++		N		N		ND		++	
Vdu1	5[nit1]		++		++		N		–		ND		++
	15[NitM]	++		++		N		N		ND		++	
FY3	65[nit1]		N		N		N		N		N		N
	34[NitM]	–		N		N		N		N		N	N
Vde1	12[nit1]		N		N		N		N		N		N
	33[NitM]	N		N		N		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, –; sparsely limited formation of complementary colonies. N: no reaction. ND; tester isolate reverted to wild-type growth. Blank; not tested.

subgroup J2 showed weak or no reaction with testers of VCG 2B, except Vdt110. As Vdt 110 was compatible with subgroup J2 and also with the tester of VCG 2B, it is possible to assign Vdt 110 to VCG 2A/B. Isolate FF1 was compatible with the tester of VCG 4A/B and showed weak reaction with the tester of VCG 4A, but no reaction with the tester of VCG 4B. In contrast, isolate Vdt9 was compatible with the tester of VCG 4A and weakly compatible with the tester of VCG 4A/B. It showed no reaction with the tester of VCG 4B. Isolates Vdt12 and Vdt102 showed weak reaction only with the tester of VCG 4A/B. Isolate FY2 showed weak reaction only with the tester of VCG 4B.

Isolates of subgroup J3 showed no or weak reaction with testers of VCG 2A, except Vdf1. Isolate Vdf 1 was compatible with subgroups J1 and J3, but showed only weak reactions with one of the testers of subgroup J2 (Table 3). Pairings among miscellaneous isolates showed compatible reaction with isolates of subgroup J3, but not with those of subgroup J2 (Table 3). As Vdf

1 was compatible with testers of VCGs 2A and 2B, it is possible to assign Vdf 1 to VCG 2A/B. For testers of VCG 2B, 9 out of 22 isolates were compatible, and the remaining isolates showed weak or no reaction. In terms of virulence of isolates, that is, 'soybean pathotype' (Suwa et al., 1987; Ebihara et al., 1999) and pathotype E in the narrow sense (Ebihara et al., 1999), 7 isolates from green soybean, but not So27, and 3 isolates from Udo (U68, U90, and U95) showed very limited reactions with the testers of VCG 2B. Isolate Vdp-4 was compatible with the testers of VCGs 2B and 4A but not with those of VCGs 4B and 4A/B.

Isolates provisionally assigned to the new subgroup J4 showed limited compatible reactions with the testers of VCG 4, although isolates of subgroup J4 did not show any reaction with testers of other VCG groups. Isolate FY3 was compatible with testers of both VCGs 4A and 4B. Isolate FY3 also showed sparse and limited reactions with VCG 2A. Isolate HR1 showed sparsely limited colony formation with testers of both VCGs 4A and

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

	FN1		FN2		FT1		FY2		Vde4		22201		Vdc4		Vdf1		Vdu1		FY3		Vde1		HR1	
	44	33	94	79	17	5	109	115	42	9	7	19	27	23	14	4	5	15	65	34	12	44	4	1
	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)
FF1	32[ <i>nit1</i> ]		++	++	++	+	++		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	42[NitM]		++	++	++	+	++		-	-	N	N	-	N	N	-	N	N	-	N	N	N	N	N
FN1	44[ <i>nit1</i> ]			++	++	++	++		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	33[NitM]			++	++	++	++		N	+	-	N	N	N	N	N	N	N	N	N	N	N	N	N
FN2	94[ <i>nit1</i> ]				++	+	++		N	-	-	N	N	N	N	N	N	N	N	N	N	N	N	N
	79[NitM]				++	++	++		N	-	-	N	N	N	N	N	N	N	N	N	N	N	N	N
FT1	17[ <i>nit1</i> ]					+	+		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+
	5[NitM]					++	++		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
FY2	109[ <i>nit1</i> ]						+		N	-	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	115[NitM]						++		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Vde4	42[ <i>nit1</i> ]								N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N
	9[NitM]								N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N
22201	7[ <i>nit1</i> ]											++	++	++					N	N	N	N	N	N
	19[NitM]											++	++	++					N	N	N	N	N	N
Vdc4	27[ <i>nit1</i> ]															++	++		N	N	N	N	N	N
	23[NitM]															++	++		N	N	N	N	N	N
Vdf1	14[ <i>nit1</i> ]																	++	N	N	N	N	N	N
	4[NitM]																	++	N	N	N	N	N	N
Vdu1	5[ <i>nit1</i> ]																			N	N	N	N	N
	15[NitM]																			N	N	N	N	N
FY3	65[ <i>nit1</i> ]																							N
	34[NitM]																				++	-	-	-
Vde1	12[ <i>nit1</i> ]																							-
	44[NitM]																							N

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



Table 5. (Continued)

VCG		1		2A				2B				3		4A		4B		4A/B					
		T9		V44		PH		WM		115		S92	70-21		PCW		BB		S39		CW		
		(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	
To26	14[nit1]		N		N		N		N		-			N			N		N		N		N
	16[NitM]	N		N		++	N	-	-	-	N	-	N		N	N	N	N	N	N	N	N	N
Vdb6	21[nit1]		N		N		N		N		N			N			N		N		N		N
	31[NitM]	N		N		++	N	N	N	-	N	N	N		N	N	N	N	N	N	N	N	N
Vdt6	23[nit1]		N		N		N		N		N			N			N		N		N		N
	27[NitM]	N		N		++	N	-	+	-	-	-	N		N	N	N	N	N	N	N	N	N
Vdt7	40[nit1]		N		N		N		N		N			N			N		N		N		N
	53[NitM]	N		N		++	N	++	-	-	-	N	N		N	N	N	N	N	N	N	N	N
Vdt9	10[nit1]		N		N		N		-		N			N			N		N		-		
	7[NitM]	N		N		++	N	N	-	N	N	N	N		N	N	++	N	N	N	N	N	N
Vdt12	8[nit1]		N		N		N		-		-			N			N		N		N		N
	6[NitM]	N		N		++	N	-	N	-	N	-	N		N	N	N	N	N	N	N	N	-
Vdt102	6[nit1]		N		N		N		N		+			N			N		N		-		
	16[NitM]	N		N		++	N	N	+	-	-	-	N		N	N		N		N		N	
Vdt103	7[nit1]		N		N		N		N		-			N			N		N		N		N
	5[NitM]	N		N		N	N	N	N	N		N	N		N	N		N		N		N	
Vdt110	1[nit1]		N		N		N		N		N			N			N		N		N		N
	25[NitM]	N		N		N	N	N	N	-	++	N	N		N	N	N	N	N	N	N	N	
J3 AC406	14[nit1]		N		N		N		N		+			N			N		N		N		N
	18[NitM]	N		N		N		N		N		N	N		N	N		N		N		N	
SM321	27[nit1]		N		N		N		N		N			N			N		N		N		N
	30[NitM]	N		N		N		N		-		-	N		N	N		N		N		N	
So1	23[nit1]		N		N		N		N		-			N			N		N		N		N
	19[NitM]	N		N		N		N		N		+	N		N	N		N		N		N	
So22	4[nit1]		N		N		N		N		N			N			N		N		N		N
	8[NitM]	N		N		N		-		-		-	N		N	N		N		N		N	
So23	17[nit1]		N		N		N		N		-			N			N		N		N		N
	1[NitM]	N		N		N		N		N		N	N		N	N		N		N		N	
So27	3[nit1]		N		N		N		N		++			N			N		N		N		N
	2[NitM]	N		N		N		N		N		N	N		N	N		N		N		N	
So28	17[nit1]		N		N		N		N		-			N			N		N		N		N
	10[NitM]	N		N		N		N		N		-	N		N	N		N		N		N	
So39	20[nit1]		N		N		N		N		N			N			N		N		N		N
	2[NitM]	N		N		N		N		N		-	N		N	N		N		N		N	
So40	2[nit1]		N		N		N		N		N			N			N		N		N		N
	14[NitM]	N		N		N		N		N		-	N		N	N		N		N		N	
So41	18[nit1]		N		N		N		N		N			N			N		N		N		N
	17[NitM]	N		N		N		N		N		-	N		N	N		N		N		N	
U54	7[nit1]		N		N		N		N		-			N			N		N		N		N
	10[NitM]	N		N		N		N		-		++	N		N	N		N		N		N	
U68	18[nit1]		N		N		N		N		N			N			N		N		N		N
	19[NitM]	N		N		N		N		-		-	N		N	N		N		N		N	

Table 5. (Continued)

VCG		1		2A				2B		3		4A		4B		4A/B							
		T9		V44		PH		WM		115		S92		70-21		PCW		BB		S39		CW	
		(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)	(1)	(M)
U69	38[nit1]		N	N		N	N				N		N		N		N		N		N		N
	60[NitM]	N		N		-	N		N		++	N		N	N		N		N		N		N
U90	7[nit1]		N	N		N	N		N				N		N		N		N		N		N
	3[NitM]	N		N		N	N		-		-	N		N	N		N		N		N		N
U95	10[nit1]		N	N		+	N		N				N		N		N		N		N		N
	11[NitM]	N		N		N	N		-		N	N		N	N		N		N		N		N
U115	6[nit1]		N	N		N	N			++			N		N		N		N		N		N
	5[NitM]	N		N		N		-		-		+	N		N	N		N		N		N	
22201	7[nit1]		N	N		N	N			++			N		N		N		N		N		N
	19[NitM]	N		N		N		-		-		++	N		N	N		N		N		N	
Vdc4	27[nit1]		N	N		N	N			++			N		N		N		N		N		N
	23[NitM]	N		N		N	N		+		-	N		N	N		N		N		N		N
Vdf1	14[nit1]		N	N		N	N			++			N		N		N		N		N		N
	4[NitM]	N		N		N	++		++		++	N		N	N		N		N		N		N
Vdp-2	5[nit1]		N	N	N	N	N	N	N	N	N		N		N		N		N		N		N
	8[NitM]	N		N		N	N	N	N	N	N		N	N		N	N		N	N		N	N
Vdp-4	3[nit1]		N	N		N	N			N			N		N		N		N		N		N
	16[NitM]	N		N		N	N	N	N	++	N	N		N	N	++	N	N		N	N		N
Vdu1	5[nit1]		N	N		N	N			++			N		N		N		N		N		N
	15[NitM]	N		N		N	+		+		++	N		N	N		N		N		N		N
J4 FY3	65[nit1]		N	N		N	N			N			N		++		++		++		++		++
	34[NitM]	N		N		-	N		N		N	N		N	++		N		-		-		-
HR1	4[nit1]		N	N		N	N			N			N		-		-		-		-		-
	1[NitM]	N		N		N	N		N		N	N		N	N		N		N		N		N
Vde1	12[nit1]		N	N		N	N			N			N		N		++		++		+		+
	44[NitM]	N		N		N	N		N		N	N		N	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; -, sparsely limited formation of complementary colonies. N, no reaction. Blank, not tested or tester isolate reverted to wild-type growth.

4B. Isolate Vde1 was compatible with the tester of VCG 4B. Although FY 3 and Vde 1 complemented each other (Table 4), the compatibilities of these two isolates were different with testers of the three subgroups of VCG 4. As the reactions of HR 1 were quite weak for all combinations, its VCG assignment will require further study.

In these compatibility tests, the testers of VCG 1 and VCG 3 showed no reactions when paired with the Japanese isolates.

## Discussion

In our previous studies, *nit 3* mutants were generated from just two isolates, HR2 and U90 (Ebihara et al., 1999). In the present study, *nit 3* mutants were generated from five isolates and the proportion of *nit 3* mutants generated among all *nit* mutants ranged from

1.6–18.2%. Some isolates of race 2 of tomato wilt generated *nit 3*, but others did not (Table 2). In terms of vegetative compatibility, two of these *nit 3* generating isolates, FY3 and Vde 1, were assigned to VCG 4. The remaining isolates were assigned to VCG 2 rather than to VCG 4. Generation of *nit 3* was correlated with neither virulence to tomato nor VCGs. *Nit 3* mutants were frequently recovered from wild-type strains of *Fusarium oxysporum* and *F. moniliforme*. The frequency of recovery of each phenotypic class of *nit* mutants on chlorate-containing media can be influenced by the nitrogen source used (Cove, 1976; Klittich and Leslie, 1988). *Nit 3* mutants were obtained by the use of asparagine, uric acid or glutamate in the medium from *Aspergillus nidulans* and *F. moniliforme*. However, *nit 3* mutants were not frequently isolated in *V. dahliae* (Chen, 1994; Daayf et al., 1995; Joaquim and Rowe, 1991; Korolev



and Katan, 1997; Strausbaugh, 1993; Strausbaugh et al., 1992). The culture age and storage conditions also affected the proportions of *nit* mutants, especially *Nit M* mutants (Korolev and Katan, 1997). The reason for our failure to obtain *nit 3* mutants remains unclear.

We conducted complementation tests on 56 Japanese isolates with the standardized tester strains of VCGs *sensu* Joaquim and Rowe (1990, 1991).

As most isolates of subgroup J1 showed strong reaction with testers of VCGs 2A and 2B, we propose that subgroup J1 be assigned to a new category, VCG 2A/B.

Most isolates of subgroup J2 complemented testers of VCG 2A. Subgroup J2, except Vdt 110, could be assigned to VCG 2A, as these isolates were highly compatible with other members of this subgroup (Ebihara et al., 1999; Nagao et al., 1997, 1998; Wakatabe et al., 1997). The mode of compatibility varied among different testers of VCG 2A. Compatible reactions of PH with isolates of subgroups J1 and J2 were quite different from those of WM (Table 5). Isolates CS-1 and PH were previously reported to show weak compatibility with isolates WM, TC, and CA in VCG 2A (Strausbaugh et al., 1992). One isolate, Vdt 7, compatible with tester WM, was also compatible with tester PH. Six isolates of subgroup J2 (U108, Vde 4, FN 1, FN 2, FT 1 and FF 1) were compatible only with tester PH. Another eight isolates of subgroup J2 (To 22, To 24, To 26, Vdb 6, Vdt 6, Vdt 9, Vdt 12 and Vdt 102) were compatible with tester PH and showed weak reactions with tester WM and also with testers of VCG 2B. Weak reactions may be worth considering in terms of subdivision of VCG 2A. However, as a strong positive reaction (++) was used as the criterion for assignment of Japanese isolates to standardized VCGs, the subdivision of VCG 2A was not considered in these experiments.

Assignment to subgroup J3 was made with hesitation because of the small number of compatible reactions with testers. As isolates of subgroup J3 were highly compatible with other members of this group (Wakatabe et al., 1997; Nagao et al., 1998; Ebihara et al., 1999), all isolates except Vdf 1 were assigned to VCG 2B. In the case of isolate Vdf 1, further study will be required because it showed strong reaction with one of the testers of subgroup J2, it was compatible with both testers of VCGs 2A and 2B, and its pathotype has not yet been determined. We did not observe weak reactions within VCG 2 of enough significance to recognize differentiation among J3 isolates.

Subgroup J4 is assigned to two subgroups: 4B for Vde 1, and VCG 4A/B for FY 3 and HR 1. These three isolates confirmed the existence of a second VCG in Japan. Rowe (1995) concluded that VCG diversity within *V. dahliae* appears to be fairly low. Japanese isolates of *V. dahliae* were also accommodated in these few VCGs. Isolates of VCG 4 were considered to be potato pathotype (Corsini et al., 1985), and those of VCG 4A were more virulent than those of VCGs 4B and 4A/B (Joaquim and Rowe, 1991; Strausbaugh, 1993; Strausbaugh et al., 1992). Furthermore, tuber yield was

reduced by the root-lesion nematode *Pratylenchus penetrans* interacting synergistically with VCG 4A isolates but not with VCG 4B isolates (Botseas and Rowe, 1994). As pathogenicity or virulence to potato was not examined for Japanese isolates, including subgroup J4, the relationship of VCGs with potato pathotype is unknown.

Several isolates of VCG 2 showed limited or strong reactions with the testers of VCG 4. Strausbaugh et al. (1992) reported weak heterokaryotic reactions between testers from VCGs 2 and 4. In our compatibility tests, weak heterokaryotic reactions were not generally observed among Japanese isolates (Table 5). These isolates may be "bridging strains" (Katan et al., 1991; Rowe, 1995), in which complete genetic isolation has not yet developed. These isolates of "bridging strains" were not concentrated in a specific pathotype but seemed to be related to the tomato pathotype, including the tomato wilt race 2 (Table 1). A single vegetative compatibility group is assigned by anastomosing of fungal strains and formation of heterokaryons by combining the strains (Bayman and Cotty, 1991; Brooker et al., 1991; Kuhlman and Bhattacharyya, 1984; Leslie, 1993; Puhalla, 1985). Anastomosis and heterokaryosis seem to be the sole means of exchanging genetic information in imperfect fungi. Even with heterokaryosis, genetic information exchange is still limited to within a certain restricted VCG. Hence, the significance of "bridging strains" in *V. dahliae* may be global exchange of genetic information between different VCGs. These "bridging strains" were compatible with VCGs 2 and 4A, while Japanese isolates of VCG 4 were assigned to 4B or 4A/B. The population of Japanese isolates of VCG 4 may be localized or quite small. In addition to these results, isolates which were compatible with both VCGs 2A and 4B or 4A/B showed weak reactions. "Bridging strains" are thought to play an important role in *V. dahliae*, although the significance of "bridging strains" in the Japanese population is not deductively obvious within the present population. Subdivision of VCG 4 occurred in the United States (Strausbaugh, 1993). It is possible that "bridging strains" in Japanese isolates are intermediates in the differentiation of subgroups of VCG 4. VCG 3 was reassigned to VCG 4A (Strausbaugh et al., 1992). However, Japanese isolates did not show any reaction with testers of VCG 3.

From these results, we concluded that Japanese isolates were composed of two VCGs, 2 and 4, "bridging strains" compatible with these VCGs, and some self-incompatible isolates.

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