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Vegetative compatibility relationships among weakly pathogenic isolates (pathotype E) of *Verticillium dahliae*

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Twenty-two isolates of *Verticillium dahliae*, which were isolated from green soybean (*Glycine max*), udo (*Aralia cordata*), horseradish (*Cochlearia armoracia*), sweetpea (*Lathyrus odoratus*), or a weed (*Chenopodium album*) were used in this study. Conidia and microsclerotia of these isolates were morphologically identical with those of *V. dahliae* but did not coincide with *V. longisporum*. Pathogenicity tests showed that these isolates were of weak pathotype. Eleven of the 22 isolates, which were obtained from green soybean and udo, were pathogenic to green soybeans. Thus pathotype E was composed of two groups: 'soybean pathotype' which was pathogenic to green soybeans; and isolates nonpathogenic to green soybeans. The latter were defined as isolates of pathotype E in the narrow sense. Selected representative *nit1* and NitM mutants of each *V. dahliae* isolate were paired with VCGJ testers. Fourteen isolates of *V. dahliae* (So1, So22, So23, So27, So28, So39, So40, So41, U54, U68, U69, U90, U95, and U115) showed complementary reactions with subgroups J1 and J3 and were assigned to subgroup J3. Isolate U108 was assigned to subgroup J2. Isolate HR1 was not compatible with any testers of VCGJ. With this exception, isolates of pathotype E in the narrow sense and those of 'soybean pathotype' were thus assigned to known VCGJ subgroups and did not form a unique group corresponding to their pathotype. 'Soybean pathotype' could not be distinguished among isolates of pathotype E by vegetative compatibility.

Key Words——*nit*; pathotype; soybean; VCG; *Verticillium dahliae*.

Verticillium dahliae Kleb. causes vascular wilt in different kinds of crops, trees, and even weeds (Hagiwara et al., 1986, 1987). This fungus seems not to differentiate strictly among host plants, in contrast to the well-known vascular wilt fungus *Fusarium oxysporum* Schlecht. (Glass and Kuldau, 1992). However, Iijima (1983) proposed the two exclusive pathotypes of *V. dahliae* based on the pathogenicity to susceptible tomatoes. These two pathotypes were further separated into five (A, B, C, D, and E) using a set of differential host plants (eggplant, pepper, tomato, and turnip) (Hagiwara, 1990; Horiuchi et al., 1990a). Although isolates of pathotype E showed less pathogenicity (faint yellowing and/or little discoloration in the vascular bundle) on the differential host plants, some of these isolates seriously affected green soybean (*Glycine max* Merr.), udo (*Aralia cordata* Thunb.), Nihon-touki (*Angelica acutiloba* (Sieb. et Zucc.) Kitagawa) (Wakatabe et al., 1990), horseradish (*Cochlearia armoracia* L.), and the weed (*Chenopodium album* L.) (Hagiwara et al., 1987). Among these host plants, green soybeans are economically important, and thus pathogenic isolates from soybeans are of special interest and have been grouped as 'soybean pathotype' (Suwa et al., 1987). Hagiwara (1990) concluded that isolates of 'soybean pathotype' could be classified as either pathotype A or E, as determined by inoculation on a set of differential host plants. However, some isolates of pathotype D, *V. longisporum* (C. Stark) Karapapa, Bainbr. & Heale (Karapapa et al., 1997), were also reported to cause severe wilting of green soybeans (Horiuchi et al., 1990b). In addition, with new differential host plants (parsley or Chinese lantern) for the pathogenicity test, a new categorization would be expected to result (Iijima, 1983; Takeda et al., 1988). The differential host method gives some information about the pathotype but is not a definitive classification method.

Pathotype recognition of *V. dahliae* was studied by vegetative compatibility using microsclerotial color mutants, and this method was successful in distinguishing defoliating strains of *V. dahliae* from VCGs of non-defoliating strains in cotton (Puhalla, 1979). These color mutants were insufficient for categorizing VCGs of Japanese isolates in our preliminary study (Nagao et al., 1994). Analysis of nitrate-nonutilizing (*nit*) mutants led

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Table 1. Origin of isolates of *Verticillium dahliae* and *V. longisporum* and their morphology of conidia and microsclerotia (MS).

Isolate	Source	Provider[a]	Locality	Length of conidia (μm)		Size of microsclerotia (μm)	Ratio of length to width of MS
				Range	Mean		
LE103	Tomato	1	Tokyo	2.5–11	4.9	34–118×30–92	1.3
So1	Green soybean	4	Gunma	2–10	4.6	40–108×31–82	1.3
So3	Green soybean	4	Gunma	3–10	5.4	32–100×23–86	1.4
So22	Green soybean	4	Gunma	2–12.5	4.9	25–88×25–84	1.3
So23	Green soybean	4	Gunma	3–12.5	5.2	50–114×40–87	1.3
So27	Green soybean	4	Gunma	2.5–10	4.9	26–91×20–54	1.3
So28	Green soybean	4	Gunma	2.5–8	4.5	38–132×30–78	1.5
So38	Green soybean	4	Gunma	2.5–11.5	4.6	35–118×27–63	1.4
So39	Green soybean	4	Gunma	2–8	4.5	44–125×35–73	1.4
So40	Green soybean	4	Gunma	2–10	4.7	20–94×19–50	1.5
So41	Green soybean	4	Gunma	2–8	4.4	23–123×20–50	1.5
U54	Udo (*Aralia cordata*)	4	Gunma	2–8	4.4	30–100×22–59	1.6
U68	Udo (*Aralia cordata*)	4	Gunma	2–9.5	4.9	35–145×25–84	1.4
U69	Udo (*Aralia cordata*)	4	Gunma	2–11	4.7	44–128×24–85	1.5
U90	Udo (*Aralia cordata*)	4	Gunma	2–9	4.7	34–180×22–70	1.7
U93	Udo (*Aralia cordata*)	4	Gunma	2–9	4.7	35–85×30–58	1.3
U95	Udo (*Aralia cordata*)	4	Gunma	2–8	4.4	35–70×21–50	1.4
U108	Udo (*Aralia cordata*)	4	Gunma	3.5–12	5.8	35–102×30–70	1.4
U115	Udo (*Aralia cordata*)	4	Gunma	2–9	4.6	38–107×32–74	1.4
HR1	Horseradish	2	Hokkaido	2.5–10.5	4.8	45–100×35–74	1.4
HR2	Horseradish	2	Hokkaido	2.5–10	4.9	30–77×23–65	1.3
88213	Sweetpea	5	Okayama	2–6.5	4.2	35–85×22–58	1.3
86101	*Chenopodium album*	3	Gunma	2–10	4.6	42–100×36–63	1.4
84010[b]	Chinese cabbage	3	Nagano	4–12.5	7.2	65–330×25–165	1.8
84111[b]	*Senecio vulgaris*	3	Nagano	4–12	7.3	50–300×45–200	2.3

a) 1=T. Iijima, 2=M. Koike, 3=H. Hagiwara, 4=T. Shiraishi, 5=S. Kasuyama.
b) *Verticillium longisporum*.

to a reassessment of the VCGs of *V. dahliae* isolates which were previously defined by Puhalla and Hummel (1983), and the number of distinct VCGs was reduced from 16 to 4 (Joaquim and Rowe, 1990). A correlation was demonstrated between genetic relatedness, based on VCG, host range, and virulence (Daayf et al., 1995; Joaquim and Rowe, 1991). Japanese isolates of *V. dahliae* formed a VCG with 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, and included race 2 of tomato wilt (Nagao et al., 1997; Wakatabe et al., 1997). There was a close relationship between pathogenicity to tomato and subgroup J2. Isolates which were not pathogenic to tomato (eggplant pathotype (A) and pepper pathotype (C)) were categorized either as subgroup J1 or subgroup J3, respectively. An isolate of pathotype E, So-2, was assigned to subgroup J1 (Wakatabe et al., 1997). Data are not sufficient to clarify a correlation between genetic relatedness, based on VCG, host range, and virulence in pathotype E.

In this study, we examined vegetative compatibility using *nit* mutants among Japanese isolates of pathotype E of *V. dahliae* and testers of the Japanese vegetative compatibility group to evaluate the significance of pathotype E as an independent group.

Materials and Methods

Isolates The origins of 25 isolates of *V. dahliae* and *V. longisporum* examined in this study are listed in Table 1. Isolates were cultured on potato-sucrose agar at 25°C in darkness.

Morphology As some isolates of pathotype D, *V. longisporum* (Karapapa et al., 1997), were reported to be pathogenic to green soybean (Horiuchi et al., 1990b), the morphology of conidia and microsclerotia of 22 isolates of *V. dahliae* was compared with that of *V. dahliae* (isolate LE103) and *V. longisporum* (isolates 84010 and 84111) (Table 1). Lengths of 200 randomly selected conidia were measured after 10–14 d of incubation. Lengths and widths of 50 microsclerotia of all isolates were measured after 10–14 d of incubation.

Pathogenicity test The inoculum was prepared as previously described (Nagao et al., 1997). Pathogenicity

tests were conducted using a set of differential host plants (eggplant cv. Senryo-Nigo, pepper cv. Ace, tomato cv. Ponderosa, and Chinese cabbage cv. Taibyo-Rokudyu-Nichi) to determine the pathogenicity groups (Horiuchi et al., 1990a). In our study, Chinese cabbage was used as host plant in place of turnip (Horiuchi et al., 1990a) and green soybean cv. Yukimusume was added to recognize 'soybean pathotype' in the pathogenicity test. Six seedlings of each host were inoculated with each isolate in October, 1997. Both external (foliar damage) and internal symptoms, the degree of vascular discoloration of root or hypocotyl, were evaluated as previously described (Nagao et al., 1997). To re-isolate inoculated pathogens, 10-mm sections of hypocotyl of all standings were sampled, stripped of the epidermis, and sterilized with 5% (v/v) sodium hypochloride solution for 5 min. After rinsing in sterilized distilled water, disinfected hypocotyls were placed on acidified water agar (1.5% (w/v)) and cultured at 25°C for 7–21 d in darkness in an attempt to isolate the pathogen.

Analysis of variance (ANOVA) among the isolates was performed with a significance level of $P=0.05$.

Recovery and characterization of *nit* mutants *Nit* mutants were recovered by a modification of the method of Puhalla (1985). Incubation of plates for 21–28 d at 23°C generated more *nit* sectors than incubation at 25°C (Wakatabe et al., 1997). Vegetative compatibility was evaluated as previously described (Wakatabe et al., 1997). Phenotype was determined from the 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 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, 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

Morphology Conidial length of isolates of pathotype E ranged from 2 to 12.5 μm (Table 1). The mean lengths of the isolates were 4.2–5.8 μm, markedly different from those of *V. longisporum* 84010 and 84111, (7.2–7.3 μm). The size range of microsclerotia was also wide, 20–180 × 19–86 μm for pathotype E and 50–330 × 25–200 μm for *V. longisporum*.

Pathogenicity test Pathogenicity of *V. dahliae* was estimated on the basis of two criteria: external and internal symptoms (Table 2). Most of the isolates examined showed a low pathogenicity rating, except for the internal index of eggplant. However, no differential host plant showed wilting or death, except when infected with HR1. Most isolates had no marked effect on either foliar or vascular conditions in the set of differential host plants. However, they showed significant differences in their virulence on tomato and eggplant ($P=0.05$). Isolates U68, U69, HR1, HR2, and 88213 showed faint yellowing and vascular discoloration on eggplant, but reisoltaion of the inocula was unseccessful. None of the isolates caused foliar symptoms on Chinese cabbage and pepper. Isolate 88213 was obtained from sweetpea, *Lathyrus odoratus* L., and was reported to be belonged to pathotype A (Kasuyama and Inoue, 1989). However, its pathotype was demonstrated as pathotype E by our inoculation test.

Symptoms on green soybean were found to be significantly different among isolates ($P=0.05$). Ten isolates from green soybean and an isolate U115 from *A. cordata* mildly infected green soybean.

Recovery of *nit* mutants Ratios of *nit* mutants in isolated sectors were not affected by the frequency of chlorate-resistant sectors. Four phenotypically characterized *nit* mutants were obtained, NitM, *nit1*, *nit2*, and *nit3* (Table 3). Both complementary *nit1* and NitM mutants were obtained for all isolates except So3 and HR2. *Nit2* appeared in eight isolates, So22, So23, So28, So38, So39, So40, So41, and U68. *Nit3* appeared in U90 and HR2. All *nit* mutants recovered were *nit1* in So3.

Vegetative compatibility group In selecting the complementary tester strains, four isolates, So38, U93, 88213, and 86101, were self-incompatible. Isolates So3 and HR2 did not generate complementary *nit1* and NitM (Table 3). Therefore, both *nit1* and NitM tester strains were examined in 16 of the 22 isolates of *V. dahliae* pathotype E. Compatibility among isolates of *V. dahliae* pathotype E was assessed (Table 4). Most isolates (14 isolates) were compatible with each other; only U108 and HR1, were not compatible. In the case of isolate So41, even though NitM mutants were recovered more frequently than *nit1* mutants, a stable and reliable complementary tester could not be selected. Thus, strain 17 of So41 (NitM) showed poor complementation with all of testers examined, in contrast to strain 18 of So41 (*nit1*). A 'soybean pathotype' could not be distinguished from the results of compatibility testing among the isolates of pathotype E.

We investigated compatibility of these 16 isolates with testers of the Japanese VCG (Table 5). Isolates of pathotype E, except for isolate HR1, were compatible with testers of subgroup J1. However, just eight isolates (So1, So22, So27, So28, So39, U54, U69, and U115) showed strong reaction with both testers of subgroup J1. The remaining seven isolates (So23, So40, So41, U68, U90, U95, and U108) were compatible with one of the testers. Among these imperfectly compatible isolates, U108 showed strongly or slightly compatible reaction with testers of subgroup J2 (LE103) but no compatibility with testers of J3. All isolates which were compatible with subgroup J1, except for U108, showed different reactions with testers of subgroup J3. Isolates compatible with subgroup J1 were compatible with at least one of the testers of subgroup J3, except for U95 and U108. Isolate U95 was weakly compatible with subgroup J3. Only three isolates, So1, So39, and U115, showed compatible reaction with both testers of subgroup J3. Judging from the results of complementa-

Table 2. Pathogenicity of Japanese isolates of *Verticillium dahliae* pathotype E on a set of differential plants and green soybean.

Isolate	Eggplant				Tomato				Pepper				Chinese cabbage				Soybean			
	Number of plants	External index[a]	Internal index[a]	% of reisolation	Number of plants	External index[a]	Internal index[a]	% of reisolation	Number of plants	External index[a]	Internal index[a]	% of reisolation	Number of plants	External index[a]	Internal index[a]	% of reisolation	Number of plants	External index[a]	Internal index[a]	% of reisolation
So1	6	2.3	3.7	0	6	1.0	1.2	0	6	1.0	1.5	0	6	1.0	1.5	0	6	3.8	3.8	100
So3	6	2.3	4.0	0	6	1.0	1.7	0	6	1.7	3.0	0	6	1.0	1.5	0	6	3.0	3.7	83.3
So22	6	2.5	4.0	16.7	6	1.0	1.8	50.0	6	1.5	2.8	0	6	1.0	1.3	0	6	4.0	4.0	100
So23	6	2.8	3.8	0	6	1.0	1.5	0	6	1.2	2.2	33.3	6	1.0	1.7	0	6	3.2	3.5	100
So27	6	2.3	3.8	0	6	1.0	1.5	0	6	1.2	2.8	0	6	1.0	1.3	0	6	3.8	4.0	100
So28	6	2.3	3.8	50.0	6	1.0	1.3	0	6	1.0	2.7	0	6	1.0	1.5	0	6	4.0	3.7	100
So38	6	2.3	3.6	0	6	1.3	2.3	83.3	6	1.3	2.5	0	6	1.2	1.5	0	6	4.0	3.7	83.3
So39	6	2.5	3.8	0	6	1.0	1.7	0	6	1.2	2.2	16.7	6	1.0	1.5	0	6	4.0	4.0	100
So40	6	2.7	3.8	0	6	1.5	2.2	66.7	6	1.5	2.5	0	6	1.0	1.7	0	6	4.0	3.7	100
So41	6	2.2	3.2	0	6	1.5	2.3	50.0	6	1.2	1.7	0	6	1.0	1.8	0	6	4.0	4.0	100
U54	6	2.8	3.7	0	6	1.2	1.8	16.7	6	1.5	3.0	0	6	1.0	1.3	0	6	1.7	2.5	62.5
U68	6	3.0	3.0	0	6	1.3	1.5	0	6	1.0	1.8	0	6	1.0	1.0	0	6	1.7	1.0	0
U69	6	3.0	3.7	0	6	1.2	1.5	16.7	6	1.3	2.7	0	6	1.0	1.5	0	6	1.7	2.3	16.7
U90	6	2.3	3.8	0	6	1.3	2.2	50.0	6	1.3	2.8	0	6	1.2	1.7	0	6	1.7	2.7	16.7
U93	6	2.7	3.8	0	6	1.2	1.8	0	6	1.0	2.5	0	6	1.0	1.0	0	6	1.0	1.7	33.3
U95	6	2.7	2.0	0	6	1.3	1.3	0	6	1.2	1.3	0	6	1.0	1.0	0	6	1.0	1.0	16.7
U108	6	2.7	2.3	0	6	1.2	1.7	16.7	6	1.2	1.2	0	6	1.0	1.0	0	6	1.0	1.0	0
U115	6	2.3	3.7	0	6	1.5	2.5	66.7	6	1.3	2.2	0	6	1.0	1.5	0	6	4.0	4.0	83.3
HR1	6	3.5	4.0	0	6	1.0	1.3	16.7	6	1.0	2.8	0	6	1.0	1.8	16.7	6	2.3	3.0	66.7
HR2	6	3.0	4.0	0	6	1.0	1.9	33.3	6	1.2	2.5	0	6	1.0	1.5	33.3	6	2.3	2.7	66.7
88213	6	3.0	4.0	0	6	1.0	1.7	16.7	6	1.3	2.5	0	6	1.0	1.8	0	6	1.7	3.3	66.7
86101	6	2.8	2.3	0	6	1.0	1.0	0	6	1.0	1.0	0	6	1.0	1.0	0	6	2.3	1.2	0
control	6	1.2	1.0	0	6	1.2	1.0	0	6	1.2	1.0	0	6	1.0	1.0	0	6	1.0	1.0	0
F value[b]		2.943*	11.71*			1.787*	2.925*			1.385	10.52*			0.952	2.302			68.2*	22.08*	

a) External symptom was evaluated by the following scale; 1=no foliar symptom; 2=yellowing of leaf; 3=wilt or necrosis of leaf; 4=wilt or necrosis of all leaves; 5=death of all leaves and apical bud. Internal symptom (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 the adjacent tissues; 4=browning of both vessels and adjacent tissues. Mean values of external symptoms (External index) and internal symptoms (Internal index) were caluculated based on replicates.

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

Table 3. Results of *nit* mutant generation and rates of *nit* mutant phenotypes of Japanese isolates of *Verticillium dahliae*.

Isolate	No. of colonies incubated on MMC	No. of generated sectors (%)[a]	No. of *nit* sectors (%)[b]	Phenotypes of *nit* (%)				
				nit1	NitM	*nit2*	*nit3*	other
So1	10	55(550.0)	42(76.4)	78.6	21.4	0.0	0.0	0.0
So3	10	46(460.0)	22(47.8)	0.0	100.0	0.0	0.0	0.0
So22	32	21(65.6)	15(71.4)	46.7	40.0	6.7	0.0	6.7
So23	32	42(131.3)	11(26.2)	63.6	27.3	9.1	0.0	0.0
So27	32	3(9.4)	3(100.0)	33.3	33.3	0.0	0.0	33.3
So28	32	27(84.3)	9(33.3)	55.6	11.1	22.2	0.0	11.1
So38	32	31(96.9)	16(51.6)	25.0	37.5	6.3	0.0	31.3
So39	32	30(93.8)	7(23.3)	57.1	14.3	28.6	0.0	0.0
So40	32	24(75.0)	16(66.7)	62.5	25.0	6.3	0.0	6.3
So41	32	30(93.8)	20(66.7)	5.0	85.0	5.0	0.0	5.0
U54	32	15(46.9)	12(80.0)	50.0	50.0	0.0	0.0	0.0
U68	32	31(96.9)	14(45.2)	50.0	28.6	7.1	0.0	14.3
U69	32	63(196.9)	11(17.5)	54.5	9.1	0.0	0.0	36.4
U90	32	160(500.0)	68(42.5)	61.8	32.4	0.0	1.5	4.4
U93	32	42(131.3)	6(14.3)	66.7	33.3	0.0	0.0	0.0
U95	32	26(81.3)	21(80.8)	57.1	42.9	0.0	0.0	0.0
U108	32	170(531.3)	37(21.8)	78.4	21.6	0.0	0.0	0.0
U115	32	7(21.9)	4(57.1)	75.0	25.0	0.0	0.0	0.0
HR1	32	12(37.5)	7(58.3)	71.4	28.6	0.0	0.0	0.0
HR2	32	39(121.9)	34(87.2)	23.5	0.0	0.0	64.7	11.8
88213	32	20(62.5)	17(85.0)	82.4	17.6	0.0	0.0	0.0
86101	32	20(62.5)	10(50.0)	80.0	20.0	0.0	0.0	0.0

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

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

tion testing among isolates and with testers of VCGJ, isolates of pathotype E were assigned to subgroup J3, except for U108 and HR 1. Isolate U108 was assigned to subgroup J2. Isolate HR1 was not complementary with any testers of VCG.

Discussion

Isolates of *V. dahliae* pathotype E were not pathogenic to a set of differential host plants in inoculation tests, but 11 of 22 isolates were proven to be pathogenic to green soybean. Thus pathotype E was composed of two kinds of groups: isolates of 'soybean pathotype,' pathogenic to green soybeans and isolates non-pathogenic to green soybeans. The latter isolates were defined as pathotype E in the narrow sense. As some isolates of pathotype D, *V. longisporum* (Karapapa et al., 1997), were also reported to be pathogenic to green soybean (Horiuchi et al., 1990b), the morphology of conidia and microsclerotia was examined (Table 1). The range of conidial length was very large, but the mean lengh of the conidia were markedly different from those of *V. longisporum*. The size distribution of microsclerotia was also wide. The microsclerotial size (the ratio of mean length and width) of pathotype E was distinguishable from that of *V. longisporum*. Our isolates of pathotype E, including isolates of 'soybean pathotype,' were confirmed to be *V. dahliae*.

Isolates of pathotype E in the narrow sense were categorizable into the known subgroup J3 (Table 5). These isolates did not exert or have lost their pathogenicity to a set of differential host plants (Table 2). On the other hand, these isolates seriously affected udo (*A. cordata*) or the weed *C. album*. This means that the differential host method did not necessarily reflect the essential differences such as genetical relatedness. Strausbaugh (1993) reported that subgroups within *V. dahliae* VCG4 exhibited different degrees of virulence in potato early dying. In our previous reports (Nagao et al., 1997; Wakatabe et al., 1997), we indicated that a close relationship existed between pathogenicity to tomato and subgroup J2. As isolates of 'soybean pathotype' have specific pathogenicity (Table 2), it was conjectured that these isolates might form a new subgroup of VCGJ. However, isolates of 'soybean pathotype' were compatible with subgroup J3 (Table 5). They were assignable to the known subgroup J3 instead of their pathotype, same as in the case of isolates of eggplant pathotype (A) and pepper pathotype (C) (Wakatabe et al., 1997). Weakly pathogenic or nonpathogenic isolates could be classified by the vegetative compatibility.

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

		So22		So23		So27		So28		So39		So40		So41		U54		U68		U69		U90		U95		U108		U115		HR1	
		4	8	17	1	3	2	17	10	20	2	2	14	18	17	7	10	18	19	38	60	7	3	10	11	44	24	6	65	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)	(1)	(M)	(1)	(M)	(1)	(M)
So1	23[*nit1*]		++		++		++		++		++		++		N		N		++		++		++		++		++		++		N
	19[NitM]	++		++		++		++		++		++		++		++		++		++		+		++		N		++		N	
So22	4[*nit1*]				++		++		++		++		++		N		++		++		++		++		+		N		++		N
	8[NitM]			++		++		++		++		++		++		++		++		++		+		+		N		++		N	
So23	17[*nit1*]						++		++		++		++		ND		++		++		++		++		+		N		++		N
	1[NitM]					++		++		++		++		N		++		++		++		+		+		N		++		N	
So27	3[*nit1*]								++		++		++		ND		++		++		++		++		++		−		++		N
	2[NitM]							++		++		++		++		++		++		++		+		+		N		++		N	
So28	17[*nit1*]										++		++		N		++		++		++		++		++		N		++		N
	10[NitM]								++		++		++		++		++		++		−		+		N		++		N	
So39	20[*nit1*]												++		N		++		++		++		++		+		N		++		N
	2[NitM]										++		++		++		++		++		N		−		N		++		N	
So40	2[*nit1*]														N		++		++		++		++		++		N		++		N
	14[NitM]													N		++		++		++		−		+		N		++		N	
So41	18[*nit1*]																N		++		++		++		+		N		++		N
	17[NitM]															N		N		N		N		N		N		ND		N	
U54	7[*nit1*]																		++		++		++		++		N		+		N
	10[NitM]																	+		++		++		N		N		++		N	
U68	18[*nit1*]																				++		++		++		N		+		N
	19[NitM]																			++		−		−		N		++		N	
U69	38[*nit1*]																						++		+		−		+		N
	60[NitM]																					++		++		N		++		N	
U90	7[*nit1*]																								+		−		+		N
	3[NitM]																							++		N		++		N	
U95	10[*nit1*]																										N		+		N
	11[NitM]																									N		+		N	
U108	44[*nit1*]																												N		N
	24[NitM]																											N		N	
U115	6[*nit1*]																														N
	65[NitM]																													N	

a) ++: 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.

Table 5. Results of pairings with testers of the Japanese VC subgroups of *Verticillium dahliae*.[a)]

VC subgroup		J1		J1		J2		J2		J3		J3	
		CM208		84023		ST1		LE103		AC406		SM312	
		11 (I)	1 (M)	25 (I)	8 (M)	18 (I)	28 (M)	30 (I)	13 (M)	14 (I)	18 (M)	27 (I)	30 (M)
So1	23[*nit*]		++		++		N		+		++		ND
	19[NitM]	++		++		N		N		++		++	
So22	4[*nit*]		++		++		N		−		+		ND
	8[NitM]	++		++		N		N		++		ND	
So23	17[*nit*]		++		++		N		N		N		++
	1[NitM]	+		+		N		N		N		+	
So27	3[*nit*]		++		++		−		+		N		++
	2[NitM]	++		++		N		N		N		++	
So28	17[*nit*]		++		++		N		ND		−		++
	10[NitM]	++		++		N		ND		−		+	
So39	20[*nit*]		++		++		N		ND		++		++
	2[NitM]	++		++		N		ND		++		++	
So40	2[*nit*]		++		++		N		N		N		++
	14[NitM]	+		+		N		N		N		++	
So41	18[*nit*]		++		++		N		−		−		++
	17[NitM]	N		N		N		N		N		N	
U54	7[*nit*]		++		++		N		N		++		ND
	10[NitM]	++		++		N		N		++		ND	
U68	18[*nit*]		++		+		N		N		+		ND
	19[NitM]	++		++		N		N		++		ND	
U69	38[*nit*]		++		++		N		−		++		++
	60[NitM]	++		++		N		N		ND		ND	
U90	7[*nit*]		++		++		N		−		N		++
	3[NitM]	++		+		N		N		N		++	
U95	10[*nit*]		++		++		N		N		+		+
	11[NitM]	N		−		N		N		+		N	
U108	44[*nit*]		−		−		N		++		N		N
	24[NitM]	++		+		−		++		N		N	
U115	6[*nit*]		++		++		−		ND		+		++
	65[NitM]	++		++		N		ND		++		++	
HR1	4[*nit*]		N		N		N		N		N		N
	1[NitM]	N		N		N		N		N		N	

a) ++: 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.

In case of isolate U108, pathogenicity tests indicated that it was of pathotype E (Table 2), but this isolate was assigned to subgroup J2 because it showed strong complementary reaction with the testers of subgroups J1 and J2, but slight or limited reactions with those of subgroup J3 (Table 5). It would be interesting to know whether isolate U108 has lost its pathogenicity to tomato or its vegetative compatibility is independent of its pathogenicity. If the former is the case, VCG will not be a criterion for distinguishing the pathogenicity to tomato. If the latter is the case, the relationship of VCG and pathogenicity will be the same as in the case of physiological races of *F. oxysporum* (Glass and Kuldau, 1992). Furthermore, this assumption will contradict the

relationship of pathotype of *V. dahliae* with VCG demonstrated in cotton (Daayf et al., 1995; Joaquim and Rowe, 1990; Puhalla, 1979) and also in tomato (Wakatabe et al., 1997).

We have used all known Japanese pathotypes determined by a set of differential host plants to examine the vegetative compatibility among them. Japanese isolates of *V. dahliae* constitute a VCG with three subgroups. Subgroup J2 is composed of tomato pathotype (Nagao et al., 1997, 1998; Wakatabe et al., 1997). Subgroup J3 includes all pathotypes other than tomato pathotype, plus one exceptional isolate, tomato-pepper pathotype (Nagao et al., 1998; Wakatabe et al., 1997). In this study, isolates of 'soybean pathotype' and pathotype E in the narrow sense with some exceptions were assigned to subgroup J3. This heterogeneity of subgroup J3 may be argued to reevaluate weakly compatible reaction among them (Table 4).

Isolate HR1 was self-compatible, but neither NitM nor *nit1* mutants showed much complementary reaction with representative *nit* testers of pathotype E or VCGJ. This isolate will be the fourth subgroup of VCGJ or a new VCG. It will be further investigated regarding its vegetative compatibility with other Japanese isolates and the reference strains of VCGs (Joaquim and Rowe, 1990).

We have been analyzing Japanese isolates of *Verticillium* spp. using random amplified polymorphic DNA (RAPD) in order to try to identify the pathogenicity groups of Japanese *Verticillium* isolates (Koike et al., 1995; Koike et al., 1996; Koike et al., 1997). Molecular analysis supported, in part, the results of differentiation based on vegetative compatibility groups. Tomato pathotype and non-tomato pathotype were distinguishable by RAPD, but differentiation among non-tomato pathotypes has not been achieved by this kind of analysis. Koike et al. (1996, 1997) examined two isolates of 'soybean pathotype,' 26ED and Vds-1 in pathotype A. These isolates were included in a subcluster of pathotypes A and C. We are now analyzing RAPD in isolates of 'soybean pathotype' of pathotype E.

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