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Vegetative Compatibility Groups and Pathogenicity Variation among Isolates of *Fusarium oxysporum* f. sp. *melonis*

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A total of 90 isolates of *Fusarium oxysporum* f. sp. *melonis*, the causal agent of oriental melon (*Cucumis melo* var. *makuwa*) wilt, was isolated from symptomatic tissues of oriental melon from 4 provinces in Korea. These isolates were grouped into vegetative compatibility groups (VCGs) by demonstrating heterokaryosis through complementation using nitrate nonutilizing (*nit*) mutants. No self-incompatibility was observed in any of isolates. All isolates were grouped into 3 VCGs; A, B, and C. Isolates belonging to VCG A and VCG B accounted for 87% and 91% of the fungal population collected in 1991 and 1993, respectively. As the increment of cultivation period in the same field, the proportion of isolates belonging to VCG B increased whereas that of isolates belonging to VCG A decreased. Mean virulence of a total population increased as the increment of cultivation period in the same field. Isolates belonging to VCG B showed the highest increment of virulence. These data suggest that replanting of a host plant in the same field may cause increase of virulence in the pathogens. Furthermore, virulence of *F. oxysporum* f. sp. *melonis* isolates is related to the VCGs.

Keywords **:** *Cucumis melo* L. var. *makuwa*, virulence, *nit* mutants, VCG.

Fusarium wilt of oriental melon (*Cucumis melo* L. var. *makuwa*), caused by *Fusarium oxysporum* Schlecht. f. sp. *melonis* Leach & Currence emend. Snyd. & Hans., is manifested in young and mature plants throughout melon growing regions (Lee, 1971; Namiki et al., 1992). This disease is one of the most limiting obstacles for stable melon production because the resistant cultivars have not been introduced and effective control methods are not available. Field soils were heavily infested by this pathogen through the continuous replanting of susceptible cultivars in the same field. Replanting failure has also been known as one of the most detrimental factors in the melon cultivation. A clear understanding of the pathogenic population of the fungus is required to enhance our ability to manage Fusarium wilt of oriental melon.

Puhalla (1985) described the relationship between VCG and pathogenicity of *F. oxysporum*, that is, a VCG and a forma specialis showed one by one correspondence. Since there were no sexual stage and meiotic recombination in this fungus, this correspondence implied the close relationship between the loci that determine vegetative incompatibility (*vic* or *het* loci) and virulence. Hopkins et al. (1992) reported that pathogenic population of *F. oxysporum* f. sp. *niveum* race 2, which is belonged to a single VCG, increased abruptly in the field where a susceptible watermelon cultivar was replanted continuously for more than 5 years. Futhermore, VCGs within a forma specialis significantly associated with the virulence level of the isolates in *F. moniliforme* and *F. oxysporum* f. sp. *cucumerinum* (Ahn et al., 1998; LaMondia and Elmer, 1989). These results suggested that VCG analysis might be used as an alternative indicator for virulence variations of fungal populations within *Fusarium* species.

The population of *F. oxysporum* f. sp. *melonis* comprises two groups that are distinguishable from each other on the basis of the virulence spectrum (Lee, 1971; Namiki et al., 1992); one group pathogenic on muskmelon (*C. melo* L. var. *reticulatus*) as well as oriental melon and the other group pathogenic only on muskmelon. Although extensive studies on VCG with races (Jacobson and Gordon, 1988 and 1991), virulence (Lee, 1971), and molecular classification including restriction fragment length polymorphism (RFLP) (Namiki et al., 1994) of *F. oxysporum* f. sp. *melonis* virulent on muskmelon have been conducted, little information is available on the relationship between VCG and virulence in *F. oxysporum* f. sp. *melonis* isolates pathogenic on oriental melon.

The aims of this study were 1) to investigate the genetic diversity in the respect of VCG, 2) to elucidate the effect of replanting a susceptible cultivar in the same field on the variation of pathogenicity, and 3) to determine the relationship between VCG and virulence of *F. oxysporum* f. sp. *melonis* isolates pathogenic on oriental

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melon.

Materials and Methods

Fungal isolates. All 90 isolates of *F. oxysporum* f. sp. *melonis* were collected from typical symptomatic tissues of oriental melon (cv. Eunsung) from 4 provinces of Korea, Kyonggi (5 fields), Chungnam (2 fields), Chonnam (1 field), and Kyungnam (1 field). Forty-seven and 43 isolates were recovered from the same fields in 1991 and 1993, respectively. Symptomatic tissues were surface-sterilized and were placed on Komada's Fusarium selective medium (Komada, 1975). All isolates were derived from a single microconidium. The identification of fungal isolates was conducted on the basis of mycological characteristics on potato dextrose agar and carnation leaf agar described by Nelson et al. (1983). All fields where the isolates collected were planted oriental melon for more than 5 years.

Virulence tests. Each isolate was grown in potato dextrose broth at 28°C for 4-5 days on an orbital shaker (120 rpm). The mycelial mats were removed by passing through 4 layers of cheesecloth, and the conidial concentration was adjusted to 5×10^5 conidia per ml. Virulence test of all isolates was performed on the susceptible melon cultivar 'Eunsung' VM-Tv-1 using the root dip method described by Jacobson and Gordon (1988). Plant seedlings were grown in soil mix (vermiculite : perlite : peat moss = 1 : 1 : 1) at 22-28°C for 2 weeks until the third true leaf began to emerge. The roots of seedlings were washed with running tap water to remove excess soil and trimmed to 4-5 cm. The roots were dipped in a conidial suspension for 15 min. The inoculated seedlings were transplanted into plastic pots (9 cm diameter × 8 cm depth) containing fresh soil mix and kept in the greenhouse. Disease severity was recorded at 4 weeks after inoculation using a scale 0-4, where 0 represents no disease; 1, slight chlorosis and wilting; 2, slight discoloration of vascular system and wilting; 3, severe, brown discoloration of vascular system and wilting; 4, complete wilting and plant death. Oriental melon plants inoculated with potato dextrose broth and an isolate M9209 were included in each run of virulence test for negative and positive controls, respectively. The experiments were conducted as a randomized complete block design with 5 replicates and 3 plants per replicate.

Vegetative compatibility. Nitrate nonutilizing (*nit*) mutants were generated and the phenotypes of all *nit* mutants were determined by the method of Correll et al. (1987). Stable *nit1* and NitM phenotypes from each isolates were stored in the sterile distilled water at 4°C. Before complementation tests among isolates, vegetative self-incompatibility of each isolate was examined (Jacobson and Gordon, 1988). *nit1* and NitM mutants of all 90 isolates of *F. oxysporum* f. sp. *melonis* were then paired in all possible combinations on *Fusarium* minimal media (MM) (Puhalla, 1985) and the plates were incubated at 26°C in the dark. Vegetative compatible isolates were recognized by the robust growth at the interface of the two colonies about 10 days after inoculation.

Results and Discussion

Generation and phenotypes of *nit* mutants. Prior to generate *nit* mutants from 90 isolates, 5 isolates (M9209, MKA-1, MKH-W, MCK04, and MNSH-8) were tested on MM with varying concentrations of $KClO_3$ (1.5, 2.0, 2.5, 3.0, and 3.5%). The highest frequency of NitM was obtained on MM containing 3.0% $KClO_3$. Subsequent experiments were conducted on MM containing 3% of $KClO_3$ to obtain *nit* mutants. A total of 1,884 *nit* mutants were obtained from 90 isolates of *F. oxysporum* f. sp. *melonis*. Seven to 21 *nit* mutants were obtained from each isolate. The phenotypes of the *nit* mutants were determined by their colony morphology on MM containing nitrate, nitrite, hypoxanthine, uric acid or ammonium tartrate as the sole nitrogen source. The *nit* mutants could be divided into three classes; *nit1* (a mutation of nitrate reductase structural locus), *nit3* (a mutation of nitrate-assimilation pathway specific locus), and NitM (mutations that affect the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity). The most frequent phenotype was *nit1* (46.5%), and followed by NitM (33.8%) and *nit3* (19.7%) among total 1,884 mutants (Table 1). Generation and phenotype assignment of *nit* mutants in this study showed that the ratio of NitM phenotype was relatively high compared with other previous studies. About 12% of mutants were identified as NitM in *F. oxysporum* f. sp. *melonis* isolates pathogenic on muskmelon (Jacobson and Gordon, 1988). Similar results were also described in the *F. graminearum* (Bowden and Leslie, 1992), *F. moniliforme* (Klittich and Leslie, 1988), and other Coelmycete fungus, *Colletotrichum orbiculare* (Wasilwa et al., 1993). This difference might be due to different medium composition for the generation of *nit* mutants such as different concentrations of potassium chlorate and removal of L-asparagine, and for prolonged incubation time.

Complementation tests of *nit* mutants. No self-incompatibility was observed between complementary *nit* mutants recovered from the same isolate. Rare occurrence of self-

Table 1. Frequency and phenotypes of *nit* mutants recovered from *Fusarium oxysporum* f. sp. *melonis*

Year	Number of Isolates	Phenotypes of *nit* mutants[a]		
		nit1	*nit3*	NitM
1991	47	526 (47)	211 (42)	403 (47)
1993	43	350 (43)	161 (32)	233 (43)
Total	90	876	372	636

[a]Numbers indicate the total numbers of the *nit* mutants. Numbers in parentheses indicate the number of isolates which produced each class of *nit* mutants from all isolates used.

Table 2. Geographic origin and number of isolates belonging to each vegetative compatibility groups of *Fusarium oxysporum* f. sp. *melonis*

Year	VCG	Number of isolates within each VCG				
		Kyonggi	Chungnam	Chonnam	Kyungnam	Total
1991	A	22	2	3	–	27
	B	10	1	2	1	14
	C	–	1	3	2	6
1993	A	14	4	1	1	20
	B	13	2	3	1	19
	C	–	–	3	1	4
Total		59	10	15	6	90

Table 3. Disease severity of *Fusarium oxysporum* f. sp. *melonis* isolates belonged to different vegetative compatibility groups

VCG	Disease severity (Collection year)[a]	
	1991	1993
A	2.0 ± 0.8Y	2.3 ± 0.3Y
B	2.1 ± 1.0Y	3.0 ± 0.2Z
C	2.3 ± 1.1Y	2.8 ± 0.3YZ
Total	2.1 ± 0.9	2.6 ± 0.3

[a]Disease severity was recorded at 4 weeks after inoculation using a scale 0-4, where 0 represents no disease; 1, slight chlorosis and wilting; 2, slight discoloration of vascular system and wilting; 3, severe, brown discoloration of vascular system and wilting; 4, complete wilting and plant death. Mean followed by the same letter within a row are not significantly different, using Duncan's multiple range tests ($P = 0.05$).

incompatibility was also observed in the various formae speciales of *F. oxysporum* (Correll, 1991: Hopkins et al., 1992: Katan et al., 1994: Katan and Katan, 1988: Ploetz, 1990). On the other hand, 16 isolates out of 28 were self-incompatible in the *F. graminearum* (Moon et al., 1999). This difference might be due to the existence or absence of gene(s) or mutations controlling heterokaryon self-incompatibility (Correll et al., 1987; Klittich and Leslie, 1988). It is generally understood that high frequency of self-incompatibility was observed in fungi having sexual cycle.

Based on pairing complementary *nit* mutants of all isolates, mainly with *nit1* and NitM, all isolates were grouped into 3 VCGs; A, B, and C (Table 2). VCG A and B are major groups and these 2 VCGs comprised of 80 isolates; VCG A (47) and VCG B (33). Previously, isolates of *F. oxysporum* f. sp. *melonis* pathogenic on muskmelon were grouped 2 large VCGs and seven small VCGs (Appel and Gordon, 1994; Jacobson and Gordon, 1988 and 1991). In 1991, isolates belonged to VCG A was 57% and those of VCG B and C was 30% and 13%, respectively. In 1993, on the other hand, isolates belonged to VCG A decreased to 47% and that of VCG B increased to 44%. Isolates belonged to VCG C were not found in Kyonggi province, although 59 out of 90 isolates were collected from Kyonggi province.

Table 4. Contrasts of between mean values of virulence and their standard deviations of *Fusarium oxysporum* f. sp. *melonis* isolates recovered in 1991 and 1993[a]

VCG	1991 vs. 1993	
	F	$P > F$[b]
A	9.79	0.1765
B	7.88	0.0260*
C	10.44	0.3775
Total	8.50	0.1776

[a]Virulence was the mean disease severity value. Disease severity was assessed with a 0-4 scale where 0 represents no disease; 1, slight chlorosis and wilting; 2, slight discoloration of vascular system and wilting; 3, severe, brown discoloration of vascular system and wilting; and 4, complete wilting and plant death.

[b]Probability of obtaining a larger absolute value of F ($P = 0.05$). Among the isolates obtained in 1991 and 1993, only the population belong to VCG B showed significantly different disease severity at $P = 0.05$.

Virulence tests. All 90 isolates caused disease symptom on oriental melon cv. Eunsung VM-Tv-1. Disease severity ranged from 1.2 to 3.1. Disease severity by isolates collected in 1993 was higher than that of isolates collected in 1991 (Table 3). On the other hand, variation of virulence was decreased in isolates collected in 1993. Statistical analysis revealed that virulence of isolates belonged VCG B was significantly increased in 1993 compared to that of 1991 (Table 4).

Our data indicated that continuous replanting of the same cultivar 'Eunsung' VM-Tv-1 resulted in the increment of the virulence of *F. oxysporum* f. sp. *melonis*. The increment of virulence was not due to the part of fungal population because the standard deviation of virulence decreased from 0.9 to 0.3. Similar phenomenon has also been observed in *F. oxysporum* f. sp. *cucumerinum* isolates (Ahn et al., 1998).

Effect of a successive replanting on fungal population structure. The number of isolates belonged to VCG B increased in 1993 compared to that in 1991. Furthermore, mean virulence of isolates belonged to VCG B increased significantly in 1993. Population structure of the pathogenic fungus varies when the culturing periods of the same host in the same field are extended. Under this environmental condition, a highly pathogenic population increased compared to less pathogenic ones. It has been suggested that replant failure of oriental melon and muskmelon due to *F. oxysporum* might be caused by the increment of highly pathogenic population of the fungus among soil microbial

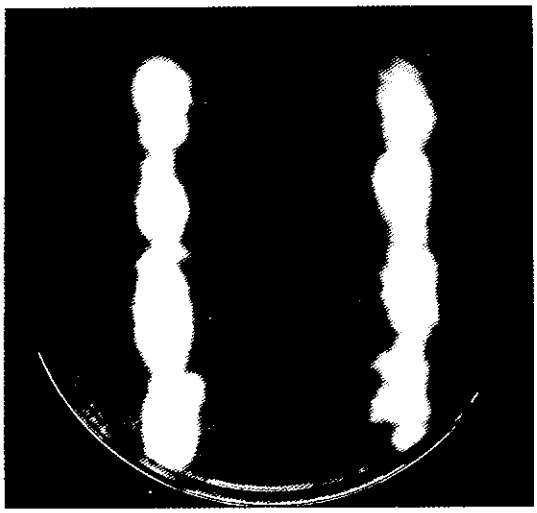

Fig. 1. Complementation tests on the *Fusarium* minimal medium containing $NaNO_3$ as the sole nitrogen source. Outer mutants (*nit1*) were generated from different isolates and paired with NitM from isolate M9209 (belonging to VCG B). Robust mycelial growth indicates complementation between isolates.

community in the field (Appel and Gordon, 1994; Lee, 1971). Similar results were observed in the *F. oxysporum* f. sp. *niveum*. Successive replanting of the susceptible cultivar resulted in the increment of the isolates of *F. oxysporum* f. sp. *niveum* race 2, which are highly pathogenic on the replanted cultivar (Hopkins et al., 1992).

Host-pathogen interactions between oriental melon and isolates of *F. oxysporum* f. sp. *melonis* have not been well established. In this report, we found a high level of correlation between a increment of virulence and replanting of the same host cultivar. Furthermore, isolates that exhibited a strong virulence belonged to the single VCG. It may be useful to monitor the outbreak of VCG B in the replanted fields for the successful control of wilt disease and to screen breeding lines of oriental melon. To our knowledge, this is the first report of the classification of *Fusarium oxysporum* f. sp. *melonis* isolates obtained from oriental melon into VCG.

References

Appel, D. J. and Gordon, T. R. 1994. Local and regional variaion in populations of *Fusarium oxysporum* from agricultural field soils. *Phytopathology* 84:786-791.

Bowden, R. L. and Resllie, J. F. 1992. Nitrate-nonutilizing mutants of *Gibberella zeae* (*Fusarium graminearum*) and their use in determining vegetative compatibility. *Exp. Mycol.* 16:308-315.

Correll, J. C., Klittich, C. J. R. and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility test. Phytopathology 77:1646-1649.

Correll, J. C. 1991. The relationship between formae speciales, races, and vegetative compatibility groups in *Fusarium oxysporum*. *Phytopathology* 81:1061-1064.

Correll, J. C., Klittich, C. J. R. and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* 77:1640-1646.

Hopkins, D. L., Lobinske, R. J. and Larkin, R. P. 1992. Selection for *Fusarium oxysporum* f. sp. *niveum* race 2 in monocultures of watermelon cultivars resistant to Fusarium wilt. *Phytopathology* 82:290-293.

Jacobson, D. J. and Gordon, T. R. 1988. Vegetative compatibility and self-incompatibility within *Fusarium oxysporum* f. sp. *melonis*. *Phytopathology* 78:668-672.

Jacobson, D. J. and Gordon, T. R. 1991. *Fusarium oxysporum* f. sp. *melonis*: a case study of diversity within a forma specialis. *Phytopathology* 81:1064-1067.

Katan, T., Berliner, R. and Katan, J. 1994. Vegetative compatibility in populations of *Fusarium oxysporum* from wild carnation. *Mycol. Res.* 98:1415-1418.

Katan, T. and Katan, J. 1988. Vegetative-compatibility grouping of *Fusarium oxysporum* f. sp. *vasinfectum* from tissue and the rhizosphere of cotton plants. *Phytopathology* 78:852-855.

Katan, T., Katan, J., Gordon, T. R. and Pozniak, D. 1994. Physiologic races and vegetative compatibility groups of *Fusarium oxysporum* f. sp. *melonis* in Israel. *Phytopathology* 84:153-157.

Klittich, C. J. R. and Leslie, J. F. 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). *Genetics* 118: 417-423.

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Rev. Plant Prot. Res.* 8:114-125.

LaMondia, J. A. and Elmer, W. H. 1989. Pathogenicity and vegetative compatibility among isolates of *Fusarium oxysporum* and *F. moniliforme* colonizing asparagus tissues. *Can. J. Bot.* 67:2420-2424.

Moon, J.-H., Lee, Y.-H. and Lee, Y.-W. 1999. Vegetative compatibility groups in *Fusarium graminearum* isolates from corn and barley in Korea. *Plant Pathol. J.* 15:53-56.

Namiki, F., Izumi, S., Kayamura, T. and Shiomi, T. 1992. Specialization of pathogenic strins of *Fusarium oxysporum* in the Cucurbitaceae. *Ann. Phytopathol. Soc. Japan* 58:540-541.

Namiki, F., Shiomi, T., Kayamura, T. and Tsuge, T. 1994. Characterization of the formae speciales of *Fusarium oxysporum* causing wilts of cucurbits by DNA fingerprinting with nuclear repetitive DNA sequences. *Appl. Environ. Microbiol.* 60:2684-2691.

Nelson, P. E., Toussoun, T. A. and Marasas, W. F. O. 1983. *Fusarium species: An illustrated manual for identification*. The Pennsylvania State University Press, University Park, PA. USA.

Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Can. J. Bot.* 63:179-183.

Wasilwa, L. A., Correll, J. C., Morelock, T. E. and McNew, R. E. 1993. Reexamination of races of the cucurbit anthracnose pathogen *Colletotrichum orbiculare*. *Phytopathology* 83: 1190-1198.