Biological and Molecular Characterization of *Fusarium oxysporum* f. sp. *lycopersici* Divides Race 1 Isolates into Separate Virulence Groups

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ABSTRACT

Mes, J. J., Weststeijn, E. A., Herlaar, F., Lambalk, J. J. M., Wijbrandi, J., Haring, M. A., and Cornelissen, B. J. C. 1999. Biological and molecular characterization of *Fusarium oxysporum* f. sp. *lycopersici* divides race 1 isolates into separate virulence groups. *Phytopathology* 89:156-160.

A collection of race 1 and race 2 isolates of *Fusarium oxysporum* f. sp. *lycopersici* was screened for vegetative compatibility and characterized by random amplified polymorphic DNA (RAPD) analysis to establish the identity and genetic diversity of the isolates. Comparison of RAPD profiles revealed two main groups that coincide with vegetative compatibility groups (VCGs). In addition, several single-member VCGs were identified that could not be grouped in one of the two main RAPD clusters. This suggests that *F. oxysporum* f. sp. *lycopersici* is a polyphyletic taxon. To assign avirulence geno-

types to race 1 isolates, they were tested for their virulence on a small set of tomato lines (*Lycopersicon esculentum*), including line OT364. This line was selected because it shows resistance to race 2 isolates but, unlike most other race 2-resistant lines, susceptibility to race 1 isolates. To exclude the influence of other components than those related to the race-specific resistance response, we tested the virulence of race 1 isolates on a susceptible tomato that has become race 2 resistant by introduction of an *I-2* transgene. The results show that both line OT364 and the transgenic line were significantly affected by four race 1 isolates, but not by seven other race 1 isolates nor by any race 2 isolates. This allowed a subdivision of race 1 isolates based on the presence or absence of an avirulence gene corresponding to the *I-2* resistance gene. The data presented here support a gene-for-gene relationship for the interaction between *F. oxysporum* f. sp. *lycopersici* and its host tomato.

Fusarium wilt caused by the fungal pathogen *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hans. is known as one of the most devastating diseases of tomato (*Lycopersicon esculentum* Mill.) worldwide (29). The three known physiological races within this forma specialis, named in order of discovery (race 1, race 2, and race 3), are distinguished by their differential pathogenicity to tomato cultivars containing race-specific, dominant resistance genes. For *F. oxysporum* f. sp. *lycopersici*, Elias and coworkers (10-12) have shown that physiological races do not correlate with vegetative compatibility groups (VCGs), which are generally considered to be evolutionary entities (20). Because both race 1 and race 2 isolates are found in the three major VCGs (VCGs 0030, 0031, and 0032), races must have evolved independently within each VCG (12).

Monogenic race-specific resistance genes against *F. oxysporum* f. sp. *lycopersici* have been identified in wild *Lycopersicon* spp. and were introgressed into commercial tomato varieties. Resistance to race 1 is governed both by gene *I* (3) and gene *I-1* (5,23), originating from accession 160 of *L. pimpinellifolium* and LA716 of *L. pennellii*, respectively. In accession PI126915 (*L. esculentum* × *L. pimpinellifolium* hybrid), resistance was found against both races 1 and 2 (1,6,26). Results of experiments by Cirulli and Alexander (6) and by Laterrot and Philouze (17) have suggested that this resistance is controlled by two separate genes. There is no genetic proof whether this race 1 resistance is allelic to the resistance of

the *I* gene or the *I-1* gene. Resistance to race 3 has been identified in accessions PI414773 and LA716 of *L. pennellii* (4,19,24).

Based on the existence of monogenic dominant resistance traits, a gene-for-gene relationship (13) for the interaction between *F. oxysporum* f. sp. *lycopersici* and tomato is generally assumed. However, a plant-fungus interaction based on the presence or absence of resistance genes in the plant and the presence or absence of corresponding avirulence genes in the fungus has never been identified. According to the gene-for-gene hypothesis, the dominant *I-2* gene in tomato would respond to a dominant avirulence gene (*AvrI-2*), present in race 2 of *F. oxysporum* f. sp. *lycopersici*. The presence or absence of *AvrI-2* in isolates of race 1, next to the avirulence gene corresponding to the plant gene conferring resistance to race 1 (*AvrI*), has never been tested because of the lack of discriminating tomato lines. In the past, such lines have not become available commercially because of the linkage of race 1 and race 2 resistance within accession PI126915 and the importance for breeders to accumulate resistance genes. However, to differentiate race 1 isolates in different avirulence pathotypes, tomato lines susceptible to race 1 but resistant to race 2 are indispensable. To our knowledge, only one such line (Ideucenzi) has been described (17). As an alternative to test for the presence of *AvrI-2* in race 1 isolates, fully susceptible lines were transformed with the *I-2* resistance gene and subsequently used in bioassays. This gene has been cloned recently and confers resistance against race 2 only (21,25).

Here, we report the characterization of a collection of isolates, the selection of a tomato line that is resistant to race 2 but susceptible to race 1, and the use of this line and a transgenic line containing the *I-2* gene to divide race 1 isolates into two groups. Results described here are consistent with the assumption that the interaction between *F. oxysporum* f. sp. *lycopersici* and tomato fits the gene-for-gene model.

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MATERIALS AND METHODS

Fungal strains. Isolates of *F. oxysporum* f. sp. *lycopersici* were obtained from several sources (Table 1).

All isolates were single-spored and stored in Protect Bacterial Preservers (Technical Service Consultants Ltd., Heywood, Lancashire, Great Britain) at -70°C .

Vegetative compatibility tests. Isolates were examined for vegetative compatibility using standard procedures (8,22). Nitrate nonutilizing (*nit*) mutants were generated, their phenotypes determined, and *nit1* and *NitM* mutants were paired. Mutants of all isolates were paired mutually and with mutants of tester strains from VCGs 0030, 0031, and 0032 (10). All isolates were assigned to a VCG based on the results of repeated pairing reactions that were incubated for at least 3 weeks.

Random amplified polymorphic DNA (RAPD) analysis. Mycelium grown in potato dextrose broth was collected, lyophilized, ground in liquid nitrogen, and stored at -20°C . The cetyltrimethylammonium bromide method described previously (20) was used for DNA isolation. Amplification reactions were performed in a total volume of 50 μl under standard conditions (31) using primers obtained from Operon Technologies, Inc. (Alameda, CA). The randomly amplified products were resolved by electrophoresis on a 1.5% agarose gel.

Profiles were transformed to binary codes on the basis of presence versus absence of amplification products of the same length. Data were analyzed using the computer program PAUP (Illinois Natural History Survey, Champaign, IL).

Plant material. Previously described (16) near-isogenic lines of tomato cultivar Moneymaker (C32, GCR161, and C295) were used as control lines. Line C32 is susceptible to both races 1 and 2; GCR161 contains the race 1 resistance of Missouri Accession 160 of *L. pimpinellifolium* (PI79532) and is susceptible to race 2; and C295 (also known as Mobox) is resistant to both races 1 and 2. In the development of this homozygous line, cultivars Walter 742-R1-3-3-BK (containing the *I-2* locus of accession PI126915) and Martarum (containing resistance to race 1 from accession PI79532) were used. However, it is not known whether both origins of race 1 resistance are still present in C295. Line Ideucenzi, resistant to race 2 but susceptible to race 1, was provided by H. Laterrot (Station d'Amelioration des Plantes Maraicheres, INRA, Avignon, France).

Crosses were made between a susceptible breeding line and different tomato lines homozygous for the *I-2* locus of accession PI126915 (derived from Florida MH1). F_2 plants were inoculated with race 2 (Fol002), and resistant plants were selfed. These F_3 lines were tested separately for resistance to race 1 (Fol004) and race 2 (Fol002). Plant lines that remained race 2 (Fol002)-resistant but susceptible to race 1 isolate Fol004 were self-pollinated for at least three generations (F_6 line) in a line selection program. Each generation was tested for susceptibility to race 1 and resistance to race 2. Finally, homozygous lines OT105, OT308, OT313, and OT364 were selected.

Susceptible tomato line KG52201 was transformed with cosmid B22 (25). This cosmid contains the full-length *I-2* gene and the C-terminal part of the *I-2C2* gene. All R_0 plants were selfed and R_1 seed tested for resistance to race 2. Transgenic lines resulted in a 3:1 segregation ratio for resistant to susceptible plants in 8 of the 11 lines tested (25). Resistant R_1 plants were selfed and R_2 seed tested for the presence of a homozygous *I-2* transgene (25). Lines KG324 and KG325, obtained from two independent primary transformants, were identified as homozygous, race 2-resistant transgenic lines.

Plant infection. Seedlings (12 days old) were infected by a standard root-dip inoculation method (30). Inoculum was prepared by growing the fungus in Czapek Dox medium at 25°C with continuous shaking at 125 rpm for 5 days. Cultures were then filtered through one layer of Miracloth (Calbiochem Corp., La Jolla, CA), centrifuged, washed with sterile water, and adjusted to a concentration of 10^7 conidia per ml. Viability of conidia was checked by plating dilutions on potato dextrose agar. Roots of uprooted seedlings were

incubated in the conidial suspension for at least 1 min and individually planted in a container (12 cm in diameter) with potting soil. In a randomized block design, the number of blocks was equal to the number of replicates (5 or 10). This resulted in equal blocks of plants, each inoculated with another isolate or water. Pots were placed with interspaces of at least 10 cm. Inoculated plants were grown at 22 to 28°C , 55 to 65% relative humidity, and 14-h light.

After 3 weeks, the degree of vascular browning was estimated and the weight of the plant part above the cotyledons was determined. Plant weight data allow the use of statistical methods appropriate for the analysis of normally distributed data. All analyses were performed per plant line. Seedlings that had clearly escaped infection ($\pm 10\%$) were excluded from our analysis.

RESULTS

Vegetative compatibility. In vegetative compatibility tests using tester strains Fol014 and Fol015, the majority of isolates could be assigned to VCG 0030 (Table 1). Isolates Fol018 and Fol019, previously classified as separate VCG 0032 (12) on the basis of results obtained after 7 days of incubation, showed compatibility when paired with several isolates of VCG 0030 after prolonged incubation (up to 21 days). This compatibility was far less pronounced than the compatibility between isolates Fol018 and Fol019. Nonetheless, these isolates should be classified within VCG 0030.

TABLE 1. Number, source, previously identified race, vegetative compatibility group (VCG), and random amplified polymorphic DNA (RAPD) groups of isolates of *Fusarium oxysporum* f. sp. *lycopersici*

Isolate	Original number	Origin and donator ^v	Race	VCG ^w	RAPD group ^x
Fol001	WCS801	Netherlands, a	1	003-***	S
Fol002	WCS862	Netherlands, b	2	0030	I
Fol003	WCS861	Netherlands, b	1	0030	I
Fol004	IPO1530	Netherlands, c	1	0030	I
Fol005	C24	Netherlands, c	2	003-*	S
Fol006		France, d	1	0030	I
Fol007		France, d	2	0030	I
Fol008		Netherlands, c	1	003-*	S
Fol009	FOL-R5-6	Wisconsin, United States, e	1	0030	I
Fol010		Netherlands, a	1	0031	II
Fol011		United States, f	1	0030	I
Fol012		Netherlands, a	1	003-***	S
Fol013		Bulgaria, g	... ^y	... ^y *	S
Fol014	LSU-3	Louisiana, United States, h	1	0030	I
Fol015	BFOL-53	Louisiana, United States, h	2	0030	I
Fol016	BFOL-51	Louisiana, United States, h	1	0031	II
Fol017	OSU-451	Ohio, United States, h	2	0031	II
Fol018	LSU-7	Louisiana, United States, h	2	0030 ^z	I
Fol019	BFOL-70	Louisiana, United States, h	2	0030 ^z	I
Fol021	Fol1	Israel, i	1	0030	I
Fol022	Fol-650 B	Israel, i	1	0030	I
Fol023	Fol-1295T	Israel, i	2	0030	I
Fol025	18947	Australia, j	2	0030	I
Fol026	14844	Australia, j	3	0030	I
Fol027	626	Florida, United States, k	1	003-***	I
Fol028	548	Florida, United States, k	2	0030	I
Fol029	5397	Florida, United States, k	3	0030	I
Fol030	218	Spain, l	1	0030	I
Fol031	4887	Spain, l	1	0030	I
Fol032	48112	Spain, l	1	003-***	I
Fol033	281	Spain, l	2	0030	I
Fol034	4287	Spain, l	2	0030	I

^v Donator is not necessarily the original collector. a = D. M. Elgersma, b = N. A. M. v Stekelenburg, c = IPO-DLO, Research Institute for Plant Protection, Wageningen, the Netherlands, d = L. Davidse; e = B. Scheffer, f = C. H. Beckma, g = Ilyeva, h = K. S. Elias, i = T. Katan, j = D. J. McGrath, k = J. P. Jones, and l = M. I. G. Roncero.

^w * = Self-compatible and ** = self-incompatible.

^x RAPD groups of isolates Fol001 to Fol019 are based on results of 19 primer OPA, remaining isolates were analyzed with OPA14 only. S = single group.

^y Not pathogenic to tomato.

^z Previously named VCG 0032.

One isolate from our collection (Fol010) could be grouped in VCG 0031 because of complementation with tester strains Fol016 and Fol017. Two self-compatible isolates (Fol005 and Fol008) did not show compatibility with any other isolate, even after prolonged incubation. Therefore, they are considered new single-member VCGs. Relatively many isolates were self-incompatible. Isolate Fol013 was not pathogenic on tomato and was included as an out-group isolate. As expected, this isolate was not vegetatively compatible with any other isolate than itself.

RAPD analysis. Patterns of randomly amplified products were established for isolates Fol001 to Fol019 with 20 random primers of Operon kit A (Operon Technologies, Inc.). Polymerase chain reactions with primer OPA-06 did not result in amplified fragments. For cluster analysis, 339 band positions were screened. This analysis revealed two distinct groups that correlated with VCGs (Fig. 1, Table 1). Self-incompatible isolates Fol001 and Fol012 and single-member VCG isolates Fol005 and Fol008 could not be assigned to either main group.

Primers OPA-02, OPA-14, and OPA-20 gave informative polymorphisms for the VCGs and different single-member isolates used in this study. These polymorphisms led to a profile that enabled us to classify isolates Fol001 to Fol019 in VCG 0030, identify them as closely related to VCG 0031, or classify them as outgroup isolates. The OPA-14 primer was chosen to classify isolates Fol021 to Fol034. All isolates displayed the characteristic pattern corresponding to VCG 0030.

Plant inoculation. Severity of symptom development, translated into an ordinal scale, is the method most commonly used to determine the susceptibility of tomato plants after infection by *F. oxysporum* f. sp. *lycopersici*. However, we found that plant fresh

weight is a good reflection of disease severity and data can be used in analysis of variance.

In initial experiments, different plant lines resistant to race 2 only were tested for their capability to differentiate race 1 isolates into two different avirulence phenotypes. Tomato lines Ideucenti, OT105, OT308, OT313, and OT364 were inoculated with a limited number of isolates of race 1. Line Ideucenti showed susceptibility to race 1 and resistance to race 2, confirming the observations by Laterrot and Philouze (17). Line OT364 was identified as a good candidate for further studies because it clearly differentiated race 1 isolates into two groups and displayed resistance to race 2; the other OT lines displayed reduced resistance to race 2 or susceptibility to all isolates of race 1.

Avirulence genotypes of race 1 isolates. To assign avirulence genotypes, OT364 and control lines were inoculated with a broad range of race 1 isolates and one representative race 2 isolate (Fol007). As expected, control line C32 was infected by all isolates tested and showed a significant reduction in plant weight (Table 2). However, not all isolates were equally aggressive. Isolates Fol008 and Fol012 were less aggressive than other isolates. Cultivar Bonny Best, a line known to be extremely susceptible to both races 1 and 2, was affected to the same extent as line C32 (data not shown). Line C295 was resistant to all isolates. The commercial variety Walter, homozygous for the accession PI 126915-derived race 1 and race 2 resistance, was tested as well. No differences in reaction were observed between this line and C295 (data not shown). In the particular experiment for which the results are shown in Table 2, race 1-resistant line GCR161 was slightly affected in weight by isolate Fol004. However, vascular browning or wilting was not observed. Line OT364 was resistant to race 2, confirming that it contains the *I-2* gene. However, it was infected by some race 1 isolates (Fol004, Fol006, Fol010, and Fol022) but not by others ($P = 0.001$), indicating the loss of race 1 resistance conferred by the *I-2* locus.

To exclude possible effects of horizontal resistance factors in line OT364 that might influence the outcome of the interaction, we used susceptible line KG52201 and the same line transgenic for the *I-2* gene in a second series of experiments. A selection of genetically closely related isolates from VCG 0030 was tested (Table 3). In contrast to susceptible line KG52201, the *I-2* transgenic lines KG324 and KG325 were completely resistant to race 2, confirming the presence of a functional *I-2* gene. The transgenic *I-2* lines were not affected significantly by race 1 isolates that were avirulent on OT364, but plant fresh weight of both lines was significantly reduced ($P = 0.001$) by the group of isolates virulent on OT364.

DISCUSSION

For many plant-pathogen interactions, race specificity of resistance can be explained by the gene-for-gene hypothesis (9,13). This

TABLE 2. Mean plant fresh weight (g) of line OT364 and three control lines 3 weeks after inoculation with isolates of *Fusarium oxysporum* f. sp. *lycopersici*

<i>F. oxysporum</i>		Tomato lines ^z			
Race	Isolate	C32	GCR161	OT364	C295
1	008	2.7 ± 2.3 b	11.5 ± 3.2 a	10.0 ± 2.8 a	13.0 ± 2.3 a
1	012	3.0 ± 2.5 b	11.6 ± 1.3 a	11.7 ± 3.0 a	13.2 ± 2.1 a
1	016	1.5 ± 1.5 c	13.1 ± 2.1 a	12.4 ± 2.8 a	13.6 ± 3.2 a
1	003	0.8 ± 0.8 cd	12.0 ± 2.6 a	9.6 ± 3.0 a	14.4 ± 3.0 a
1	001	0.4 ± 0.6 cd	13.1 ± 2.0 a	12.3 ± 2.8 a	13.5 ± 1.5 a
1	009	0.4 ± 0.5 cd	13.1 ± 2.0 a	9.6 ± 3.9 a	13.2 ± 3.0 a
1	011	0.1 ± 0.1 d	12.0 ± 2.4 a	11.3 ± 3.9 a	12.5 ± 1.6 a
1	021	0.3 ± 0.4 d	12.8 ± 2.9 a	9.7 ± 3.0 a	12.3 ± 1.8 a
1	010	0.1 ± 0.1 d	12.8 ± 3.3 a	6.3 ± 3.8 b	14.0 ± 2.1 a
1	022	0.2 ± 0.5 d	11.6 ± 2.6 a	6.0 ± 3.1 bc	13.2 ± 3.0 a
1	006	0.03 ± 0.04 d	2.2 ± 2.5 a	4.3 ± 2.9 bc	13.1 ± 1.6 a
1	004	0.2 ± 0.3 d	8.4 ± 2.1 b	3.2 ± 2.3 c	12.2 ± 3.6 a
2	007	0.1 ± 0.1 d	1.0 ± 0.9 c	11.7 ± 2.7 a	13.1 ± 1.5 a
H ₂ O	Control	11.5 ± 3.1 a	12.3 ± 2.5 a	12.3 ± 2.0 a	12.0 ± 1.8 a

^z Mean ± SD, mean is based on 10 replicates. Vertical row values followed by the same letter do not differ significantly according to the *F* test ($P = 95\%$). C32, $P = 0.001$; GCR161, $P = 0.001$; OT364, $P = 0.001$; and C295, $P = 0.4562$.

Fig. 1. Phylogenetic relationship of *Fusarium oxysporum* f. sp. *lycopersici* isolates based on random amplified polymorphic DNA (RAPD) analysis. RAPD patterns were analyzed with the computer program PAUP, using the branch and bound method. The figure is one of four most-parsimonious trees (tree length = 449).

hypothesis implies that incompatibility requires both a dominant resistance gene in the host and a corresponding dominant avirulence gene in the pathogen. In the absence of either one of the two genes, pathogen recognition does not occur, defense responses are not triggered, and disease ensues. For only a few plant-pathogen interactions, a gene-for-gene relationship has been demonstrated by inheritance analysis of the matching genes in the host and pathogen, respectively (9,28). More often, a gene-for-gene interaction has been suggested or generally assumed without complete genetic evidence, as is the case for the interaction between *F. oxysporum* f. sp. *lycopersici* and tomato. The presence of monogenic dominant resistance genes in the host has been demonstrated. Recently, gene *I-2*, conferring specific resistance to race 2, has been cloned and characterized (25). Its predicted product shares common domains found in other resistance genes of the NBS-LRR class (2,27). Other members of this class of resistance genes (including *N*, *L6*, *RPM1*, and *RPS2*) are all known to be involved in gene-for-gene interactions. Hence, it is reasonable to hypothesize that a gene-for-gene interaction exists between tomato and *F. oxysporum* f. sp. *lycopersici* as well. Classical genetic studies with the fungus are not possible because of the absence of a sexual cycle. However, if the gene-for-gene hypothesis holds true for this interaction, it should be possible to divide a natural population of race 1 isolates into two groups based on the presence or absence of a functional *AvrI-2* corresponding to the *I-2* resistance gene. The results presented here support the existence of a gene-for-gene relationship.

Isolates of *F. oxysporum* f. sp. *lycopersici* were characterized by vegetative compatibility and RAPD analysis. Studies on the genetic diversity of this fungus by VCG and restriction fragment length polymorphism (RFLP) analysis have been published (10–12). Our data support the idea that isolates within a VCG are genetically more similar, and probably derivatives of a common ancestor, than those in different VCGs, regardless of their race. Based on RFLP analyses, Elias et al. (12) concluded that VCGs 0030 and 0032 have diverged recently, because isolates from both groups often exhibited identical RFLPs. We have currently found that both VCGs are vegetatively compatible in a prolonged compatibility test. Therefore, we suggest that isolates of both VCGs be classified into VCG 0030. It is possible that the isolates of the single-member VCGs have evolved from one of the larger VCGs, but it is more likely that they are newly identified, independent entities within *F. oxysporum* f. sp. *lycopersici*. These VCGs cannot yet be given a number, because we have not been able to compare them with testers of VCG 0033, a name given to a specific group of race 3 isolates (18) not present in our collection. Relatively many isolates within the forma specialis *lycopersici* belong to single-member VCGs or are self-incompatible (10). Therefore, VCG analysis is not an optimal technique to relate new isolates to the currently described groups. RAPD analysis is a simple, fast, and more straightforward method, because all isolates can be characterized with the use of universal primers and placed into the diverse genetic population. By our study, and the one performed in the group of T. Katan (*personal communication*), a basis for such an identification by RAPDs is initiated.

To subdivide race 1 isolates, we needed tomato lines with a specific resistant genotype. We have identified line OT364 as resistant to race 2 but susceptible to some race 1 isolates. However, it retained the ability to resist other race 1 isolates (Table 2). To excluded the influence of components other than those related to the race-specific resistant response, we have used homozygous transgenic *I-2* lines to confirm our results with OT364. The results allow us to divide race 1 isolates into two groups (Table 4). Such a division fits the gene-for-gene hypothesis that predicts that resistance can only be displayed when both resistance gene and corresponding avirulence gene are present. According to this reasoning, race 1 isolates all must express a functional *AvrI*, matching the resistance gene to race 1. Race 2 isolates, being able to induce disease in plants bearing race 1 resistance, lack a functional *AvrI*. In these race 2 isolates, *AvrI-2* is postulated to account for resistance observed with line OT364, matching resistance gene *I-2*. We have found that not only race 2 isolates express *AvrI-2*, but some of the race 1 isolates do as well. Consequently, race 1 isolates avirulent on the transgenic lines have to express both *AvrI* and *AvrI-2*. Those isolate were identified in both VCG 0030 and VCG 0031. Of the 11 tested race 1 isolates, 4 are able to infect OT364 and, therefore, lack a functional *AvrI-2*. This genotype is found in both VCGs as well (Table 4).

The results support the gene-for-gene hypothesis because we have identified two groups within a group of genetically related race 1 isolates, most probably based on the presence or absence of *AvrI-2* corresponding to resistance gene *I-2* (Table 4). Whether avirulence genes *AvrI* and *AvrI-2* really exist can only be determined by the isolation of mutants, cloning and characterization of the genes; characterization of the gene products; and determination of the functional role of these products in inducing resistance.

Our evidence for the existence of a gene-for-gene interaction justifies changing the nomenclature of the races of *F. oxysporum* f. sp.

TABLE 4. Summary of interactions between tomato cultivars and isolates of *Fusarium oxysporum* f. sp. *lycopersici*^x

<i>F. oxysporum</i>				Tomato lines ^y			
VCG ^z	Isolate	Current race	Putative genotype	C32 (i i2)	GCR161 (I i2)	OT364/KG324 (i I2)	C295 (I I2)
0030	003	1	<i>AvrI AvrI-2</i>	S	R	R	R
0030	009	1	<i>AvrI AvrI-2</i>	S	R	R	R
0030	011	1	<i>AvrI AvrI-2</i>	S	R	R	R
0030	021	1	<i>AvrI AvrI-2</i>	S	R	R	R
0031	016	1	<i>AvrI AvrI-2</i>	S	R	R	R
003–	001	1	<i>AvrI AvrI-2</i>	S	R	R	R
003–	008	1	<i>AvrI AvrI-2</i>	S	R	R	R
003–	012	1	<i>AvrI AvrI-2</i>	S	R	R	R
0030	004	1	<i>AvrI –</i>	S	R	S	R
0030	006	1	<i>AvrI –</i>	S	R	S	R
0030	022	1	<i>AvrI –</i>	S	R	S	R
0031	010	1	<i>AvrI –</i>	S	R	S	R
0030	007	2	<i>– AvrI-2</i>	S	S	R	R

^x The putative avirulence genotype of the fungal isolates was inferred from the observed interactions.

^y R = resistant and S = susceptible.

^z VCG = vegetative compatibility group.

TABLE 3. Mean plant fresh weight (g) of *I-2* transgenic lines KG324 and KG325 and three control lines 3 weeks after inoculation with isolates of *Fusarium oxysporum* f. sp. *lycopersici* of VCG 0030

		Tomato lines ^z				
Race	Isolate	KG52201	GCR161	KG324	KG325	C295
1	003	0.4 ± 0.4 b	10.4 ± 1.3 a	6.6 ± 1.1 a	6.6 ± 3.1 b	10.5 ± 0.8 a
1	009	0.1 ± 0.1 b	10.1 ± 1.3 a	6.2 ± 1.5 a	7.4 ± 2.3 b	9.7 ± 1.0 a
1	011	0.1 ± 0.2 b	10.0 ± 0.9 a	7.7 ± 1.3 a	6.6 ± 3.7 b	10.7 ± 1.3 a
1	022	0.0 ± 0.1 b	9.6 ± 1.9 a	3.2 ± 3.5 b	3.5 ± 2.5 c	10.2 ± 1.1 a
1	006	0.0 ± 0.1 b	10.5 ± 1.9 a	2.9 ± 2.9 bc	2.0 ± 2.1 c	11.2 ± 1.1 a
1	004	0.0 ± 0.1 b	10.5 ± 0.9 a	1.0 ± 1.0 c	1.9 ± 2.3 c	10.9 ± 1.4 a
2	007	0.0 ± 0.1 b	1.1 ± 1.8 b	7.7 ± 1.4 a	8.8 ± 0.8 ab	10.3 ± 1.4 a
H ₂ O	Control	10.1 ± 1.3 a	10.8 ± 1.4 a	8.0 ± 1.5 a	9.8 ± 1.2 a	11.4 ± 1.7 a

^z Mean ± SD, mean is based on 10 replicates. Vertical row values followed by the same letter do not differ significantly according to the *F* test (*P* = 95%). KG52201, *P* = 0.001; GCR161, *P* = 0.001; KG324, *P* = 0.001; KG325, *P* = 0.001; and C295, *P* = 0.078.

lycopersici in a more usual gene-for-gene nomenclature, based on the absence of avirulence genes. Such a nomenclature was proposed by Gabe (14) and supported by Laterrot and Philouze (17). However, it never became current. Because of the impact it will have for plant breeders and scientists, we do not suggest to change the nomenclature, but we propose to use the supplement of virulence or avirulence genotype whenever it is important for understanding the results.

Identification of lines such as OT364 and Ideucenzi is a rare event because of the low frequencies of crossover between the tightly linked resistances to races 1 and 2 in accession PI126915. Furthermore, the presence of the *I-2* gene reduces susceptibility to isolates of race 1 (17). Such reduced susceptibility was observed with OT364 (Table 2) and the transgenic lines (Table 3). Susceptibility of OT364 and the transgenic lines resulted in 25 to 50% reduction of plant weight, whereas a susceptible interaction of cultivar Moneymaker C32 or KG52201 with the same isolates resulted in almost 100% reduction. By using the transgenic lines, we now can conclude that reduction of susceptibility was due to the *I-2* gene itself, excluding other resistance components. It is possible that besides mediating a race-specific resistance response there is another function of the *I-2* protein that contributes to a higher level of basal resistance. Alternatively, there may exist homology between the *AvrI* and *AvrI-2* gene products causing an incompletable *I-2* dependent resistance response to race 1 isolates. Antisense expression of a *I-2* homologue affected the race 2 but not the race 1 resistance, suggesting that it is not likely that the *I* resistant gene corresponding to *AvrI* has a high level of homology with the *I-2* gene (21).

In our collection of isolates, we found all avirulence types in both major VCGs, 0030 and 0031 (race 3, lacking both *AvrI* and *AvrI-2*, was found within VCG 0030 as well). Therefore, the observed virulence cannot be simply explained by independent host specialization of these VCGs. Furthermore, the accession 160-derived *I* gene (3) also confers resistance to Fol001, Fol008, and Fol012 isolates, which probably have a different origin than common VCGs 0030 or 0031. The fact that Fol008 and Fol012 seem to be less adapted to tomato (Table 2) supports this hypothesis. Likewise, the race 2 resistance from accession PI126915 (26) confers resistance to isolate Fol005 (data not shown), an isolate that appears to be of independent origin. This population structure of *F. oxysporum* f. sp. *lycopersici*, many VCGs with similar races within each VCG, raises questions about its evolutionary history. Models to explain the evolutionary relationship between formae speciales, VCGs, and races in *F. oxysporum* have been described (7,15), and several patterns are identified in the various formae speciales. For the forma specialis *lycopersici*, it is clear that the VCGs are diverse because of the observed differences in RFLP and RAPD patterns between VCGs. If VCG diversification developed before host specialization, mechanisms for virulence (pathogenicity) to tomato may be different for isolates from different VCGs. Regardless of whether host specificity or VCG diversification came first, isolates within different VCGs are expected to share characteristics because of a common ancestry. Mutations that alter virulence could have occurred within each VCG, with or without selection pressure, leading to similar races in different VCGs.

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