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Restriction fragment length polymorphisms, races and vegetative compatibility groups within a worldwide collection of *Fusarium oxysporum* f.sp. *gladioli*

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Isolates of *Fusarium oxysporum* f.sp. *gladioli* were collected from widely different geographic areas. These isolates were characterized by pathogenicity to two differential gladiolus cultivars, vegetative compatibility, and total genomic DNA restriction fragment length polymorphisms (RFLPs). RFLPs were used to estimate the genetic divergence and relationship among isolates of *F. oxysporum*. RFLPs were detected by Southern blot hybridization of total genomic DNA with a 3.4 kb DNA probe generated from total DNA of *F. oxysporum* f.sp. *dianthi*. Cluster analysis allowed the division of pathogenic strains into three main RFLP groups, each group containing strains with similarity coefficients ranging from 78 to 100%. RFLP groups correlated with vegetative compatibility groups, not with races. Two single pathogenic isolates which could not be assigned to any of the three main vegetative compatibility groups also had distinctive RFLP patterns. Little genetic polymorphism was observed within vegetative compatibility groups, whereas the majority of RFLPs occurred between vegetative compatibility groups, suggesting a common ancestry for strains within a specific vegetative compatibility group and a polyphyletic origin for the present special form *gladioli*.

INTRODUCTION

The fungus *Fusarium oxysporum* f.sp. *gladioli* (Massey, 1926; Boerema & Hamers, 1989) causes corm rot, yellows disease and shoot length reduction of gladiolus (Bald *et al.*, 1971; Nelson *et al.*, 1981) and other iridaceous plants (McClellan, 1945). We recently reported on differentiation of this special form into two races based on pathogenicity to two differential gladiolus cultivars, and on the existence of three vegetative compatibility groups (VCG 0340, 0341 and 0342). The relationship between vegetative compatibility groups and races proved to be complex (Roebroeck & Mes, 1992).

Vegetative compatibility indicates a qualitative rather than a quantitative relationship since incompatibility can be caused by single changes in one of the *vic* loci (Puhalla & Spieth, 1985). Vegetative compatibility cannot be used to determine the genetic distance between isolates of different vegetative compatibility groups and,

moreover, isolates of *Fusarium oxysporum* that are incapable of forming a heterokaryon cannot even be assigned to a vegetative compatibility group. Determination of restriction fragment length polymorphisms (RFLPs) has proved to be a powerful technique for examination of genetic variation at the DNA level, when studying the phylogenetic relationships of populations or individuals (Avisé *et al.*, 1987; Coddington *et al.*, 1987; Kistler *et al.*, 1987; Manicom *et al.*, 1987). Such studies have led to the development of polymorphic markers that can be used in the identification of isolates, linkage mapping and the identification of markers linked to loci of interest (Beckmann & Soller, 1983; Michelmore & Hulbert, 1987).

A previous study on the relationships between vegetative compatibility groups and races within the *F. oxysporum* f.sp. *gladioli* (Roebroeck & Mes, 1992) included 31, mainly Dutch, isolates. In the present study 26 additional isolates from diseased gladiolus corms, collected from widely different geographic regions, were examined. In addition, we investigated genetic variation within the special form by analysing RFLPs of all 57 isolates, encompassing the previously reported

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collection (Roebroeck & Mes, 1992). For this purpose we used a random probe generated from total DNA of an isolate of *F. oxysporum* f.sp. *dianthi* (Manicom *et al.*, 1987).

MATERIALS AND METHODS

Fungal strains

Twenty-six Dutch isolates of *F. oxysporum* and 31 others from *Gladiolus* sp. and other iridaceous plants were studied (Table 1). Part of this collection has been described previously (Roebroeck & Mes, 1992). Foreign isolates were received from South Africa (J. Ferreira, Elsenburg Agricultural Development Institute, Elsenburg), India (N. Ramachandran, Indian Institute of Horticultural Research, Bangalore), Czechoslovakia (E. Dušková, Ornamental Gardening and Plant Breeding Research Institute, Pruhonic), Australia and the USA (P. E. Nelson, Pennsylvania State University, USA). Four isolates were received from the American Type Culture Collection (ATCC), Rockville, Maryland, USA. Isolates G75 and G76 were recovered in our institute from diseased gladiolus corms from Brazil and Italy, respectively.

Pathogenicity tests

To determine the race identity of isolates of *F. oxysporum*, inoculations were performed on corms of the 'large-flowered' gladiolus cultivar Peter Pears (susceptible to race 1) and the 'small-flowered' gladiolus cultivar Nymph (susceptible to races 1 and 2). Inoculation procedures (corm dipped in a spore suspension), growing conditions and disease analysis have been described previously (Roebroeck & Mes, 1992). Five replicates with one corm per pot were used.

Vegetative compatibility tests

Isolates were examined for vegetative compatibility using standard procedures (Puhalla, 1985; Roebroeck & Mes, 1992). Nitrate-non-utilizing (*nit*) mutants were generated, their phenotypes determined and *nit1* and *NitM* mutants selected and paired. Mutants of self-compatible isolates were paired with mutants of tester strains from VCGs 0340, 0341 and 0342. All isolates were assigned to a vegetative compatibility group based on results of repeated pairing reactions. In order to find new vegetative compatibility groups, isolates that did not pair with any of the testers were paired together.

RFLP analysis

Slices from potato dextrose agar (PDA) cultures were used to inoculate liquid Czapek Dox medium. From 1-month-old stationary cultures grown at room temperature, mycelium was collected by filtration, lyophilized, ground with mortar and pestle in the presence of liquid nitrogen and stored at -20°C . DNA was isolated, as described by Manicom *et al.* (1987) using cetyltrimethylammonium bromide (CTAB), but without repeated extraction, in buffer containing 10% CTAB. Probe 'D4', a 3400 bp random *Hind*III fragment from *Fusarium oxysporum* f.sp. *dianthi*, was kindly supplied by B. Q. Manicom (Manicom *et al.*, 1987; 1990). The fragment was ligated into the *Hind*III site of pBR322 and used to transform *E. coli* HB101. Plasmid DNA containing the insert was isolated (Maniatis *et al.*, 1982), digested with *Hind*III and the D4 fragment isolated using the GeneClean II kit (Bio 101, Inc., USA). Total genomic fungal DNA was digested with *Hind*III according to the manufacturer's instructions (Boehringer Mannheim GmbH, Germany). The digests were separated on 1% agarose gels and blotted to Hybond N⁺ membranes (Amersham International plc, UK). Blots were incubated with a horseradish-peroxidase-labelled probe and treated according to standard instructions with the chemiluminescent substrate luminol (Amersham, ECL-kit). Blots were exposed to Kodak X-ray films for 10–60 min.

Experiments with DNA of isolates that did not hybridize with the probe, or yielding only scarcely perceptible bands, were repeated at least once. Isolates still yielding no or weak hybridization were not included in the cluster analysis.

Hybridization patterns on the autoradiogram were used to examine the relationships between the isolates. Bands with different electrophoretic mobility were assigned position numbers. RFLPs were transformed to binary codes by assigning a 1 to bands present or a 0 to absent bands. With the help of a GENSTAT computer program (Payne *et al.*, 1987), Sørensen similarity was calculated and dendrograms with average linkage were drawn (Digby, 1984a, b).

RESULTS

Pathogenicity tests

Results of pathogenicity tests with isolates not studied previously (Roebroeck & Mes, 1992) are given in Table 2. Most control plants either did not develop any symptoms (index 0), or devel-

Table 1. Origin, race, vegetative compatibility group (VCG) and restriction fragment length polymorphism (RFLP) group of isolates of *Fusarium oxysporum* f.sp. *gladioli*

Present code	Original code	Origin		Race ^a	VCG	RFLP group
		country	host			
G1		Netherlands	<i>Gladiolus</i> sp.	1	0340	I
G2		Netherlands	<i>Gladiolus</i> sp.	1	0340	I
G3		Netherlands	<i>Gladiolus</i> sp.	1	0340	I
G4		Netherlands	<i>Gladiolus</i> sp.	1	0340	I
G7		Netherlands	<i>Gladiolus</i> sp.	1	0340	I
G9		Netherlands	<i>Gladiolus</i> sp.	1	0340	I
G11		Netherlands	<i>Gladiolus</i> sp.	1	0340	I
G12		Netherlands	<i>Gladiolus</i> sp.	1	0340	I
G15		Netherlands	<i>G. italicus</i>	1	0340	I
G16		Netherlands	<i>Gladiolus</i> sp.	1	0340	I
G21		Netherlands	<i>Gladiolus</i> sp.	1	0340	I
G31	I A	India	<i>Gladiolus</i> sp.	1	0340	I
G32	I B	India	<i>Gladiolus</i> sp.	1	0340	I
G33	2542	Czechoslovakia	<i>Gladiolus</i> sp.	1	0340	I
G34	2543	Czechoslovakia	<i>Gladiolus</i> sp.	1	0340	I
G35	2544	Czechoslovakia	<i>Gladiolus</i> sp.	1	0340	I
G57	ATCC 26849	USA	<i>Gladiolus</i> sp.	1	0340	I
G63	0-1151	Australia	<i>Gladiolus</i> sp.	1	0340	I
G64	0-1152	Australia	<i>Gladiolus</i> sp.	1	0340	I
G67	0-0700	USA	<i>Gladiolus</i> sp.	1	0340	I
G70	0-0708	USA	<i>Gladiolus</i> sp.	1	0340	I
G71	0-0715	USA	<i>Gladiolus</i> sp.	1	0340	I
G73	0-0719	USA	<i>Gladiolus</i> sp.	1	0340	I
G74	0-0725	USA	<i>Gladiolus</i> sp.	1	0340	I
G75		Brazil	<i>Gladiolus</i> sp.	1	0340	I
Ir7		Netherlands	<i>Iris</i> sp.	1	0340	I
G19		Netherlands	<i>Gladiolus</i> sp.	1	0340	wh
G55	ATCC 11137	USA	<i>Gladiolus</i> sp.	1	034-si	I
G56	ATCC 18133	USA	<i>Gladiolus</i> sp.	1	034-si	I
G6		Netherlands	<i>Gladiolus</i> sp.	2	0340	I
G18		Netherlands	<i>Gladiolus</i> sp.	2	0340	I
G24		Netherlands	<i>Gladiolus</i> sp.	2	0340	I
G5		Netherlands	<i>Gladiolus</i> sp.	2	0341	II
G8		Netherlands	<i>Gladiolus</i> sp.	2	0341	II
G14		Netherlands	<i>Gladiolus</i> sp.	2	0341	II
G17		Netherlands	<i>Gladiolus</i> sp.	2	0341	II
G23		Netherlands	<i>Gladiolus</i> sp.	2	0341	II
Fr1		Netherlands	<i>Freesia</i> sp.	2	0341	II
Cr1		Netherlands	<i>Crocus</i> sp.	2	0342	III
Cr2	CBS 620-72	Germany	<i>Crocus</i> sp.	2	0342	III
G25	CBS 151-27	USA	<i>Gladiolus</i> sp.	2	034-si	IV
G76		Italy	<i>Gladiolus</i> sp.	1	0343	V
G10		Netherlands	<i>Gladiolus</i> sp.	-	si	VI
G13*		Netherlands	<i>G. illyricus</i>	-	-	VII
G26	CBS 253-52	Italy	<i>Gladiolus</i> sp.	-	-	VIII
G27	CBS 160-57	Germany	<i>Gladiolus</i> sp.	-	-	IX
G28	FG 6-1	South Africa	<i>Gladiolus</i> sp.	-	-	X
G29	FG 6-2	South Africa	<i>Gladiolus</i> sp.	-	-	XI
G58	ATCC 46082	Japan	<i>G. grandavensis</i>	-	si	XII
G68	0-0702	USA	<i>Gladiolus</i> sp.	-	-	XIII

Table 1. (contd)

Present code	Original code	Origin		Race ^a	VCG	RFLP group
		country	host			
G69	0-0705	USA	<i>Gladiolus</i> sp.			XIV
G72	0-0717	USA	<i>Gladiolus</i> sp.			XV
M685*		USA	<i>Gladiolus</i> sp.			XVI
G22		Netherlands	<i>Gladiolus</i> sp.			wh
G30	FG 6-2H	South Africa	<i>Gladiolus</i> sp.			nh
G65	0-0013	USA	<i>Gladiolus</i> sp.			nh
G66	0-0699	USA	<i>Gladiolus</i> sp.			nh

^a Race identification according to present data (Table 2) and Roebroeck & Mes (1992).

si, self-incompatible; *, *Fusarium proliferatum*; wh, weak hybridization; nh, no hybridization; -, tested, but could not be assigned to a group.

ATCC, American Type Culture Collection (Rockville, USA)

CBS, Centraalbureau voor Schimmelcultures (Baarn, the Netherlands).

oped some superficial browning (index 1). Corms of inoculated plants with symptoms recorded as index 1 could therefore not be considered diseased. Some corms of control plants developed disease symptoms, probably owing to the presence of latent infection, an unfortunate inevitability when using corm from commercial stocks (Roebroeck *et al.*, 1990). Because of this background level of infection, cultivars with only one or two diseased corms (index 2-4) out of the five inoculated were not considered susceptible to the isolate under test. In the few cases where this decision was required, no significant reduction of shoot length was observed while the reduction of shoot length caused by obviously pathogenic isolates was highly significant. These findings support the decision to classify isolates on this basis.

Pathogenic isolates were identified as race 1 or race 2 using two differential *gladiolus* cultivars (Roebroeck & Mes, 1992; Table 1). All newly tested pathogenic isolates belonged to race 1 (pathogenic to both 'Peter Pears' and 'Nymph'). Shoot length reduction and corm rot of 'Peter Pears' and 'Nymph' inoculated with spores of G71 were less than when inoculated with other isolates of race 1 (Table 2). The lower aggressiveness of isolate G71 is probably a result of its slow growth rate, observed when mycelium was grown on agar plates. Isolate G76 was as pathogenic to 'Nymph' as to other isolates of race 1 but 'Peter Pears' was only moderately susceptible. It is possible that G76 may represent a previously

undescribed race. Isolates from South Africa and some of those received from the USA were not pathogenic to the cultivars tested.

Vegetative compatibility tests

Column 6 in Table 1 shows the results of the vegetative compatibility tests for all isolates tested. The newly tested, pathogenic, self-compatible isolates were all restricted to VCG 0340, except for isolate G76. This self-compatible isolate appeared to be the single member of a distinct vegetative compatibility group. Two out of three pathogenic isolates obtained from the ATCC were self-incompatible. Isolates pathogenic to *gladiolus* but self-incompatible were designated VCG 034-, a code suggested by Jacobson and Gordon (1988) for this type of isolate.

The remaining, non-pathogenic isolates were either self-incompatible or self-compatible, but did not react with other isolates in complementation tests. These isolates were not assigned VCG numbers.

RFLP analysis

Most of the isolates investigated yielded RFLPs when a *Hind*III digest of total genomic DNA was probed with D4. A clustering limit at about 70% resulted in a clustering of the pathogenic isolates into three main RFLP groups (I, II and III); the two remaining isolates showed single distinctive RFLP patterns (IV and V). Results of the cluster analysis, possibly indicating the genetic relation-

Table 2. Pathogenicity of 26 isolates of *Fusarium oxysporum* f.sp. *gladioli* from different geographic origin to two differential gladiolus cultivars

Present code	Original code	Relative shoot length ^a		Corm rot disease index		Race
		PP	N	PP	N	
Control		100	100	0,4,0,0,0	0,0,0,1,1	–
G31	I A	0***	0***	4,4,4,4,4	4,4,4,4,4	1
G32	I B	1***	0***	4,4,4,4,4	4,4,4,4,4	1
G33	2542	2***	0***	4,4,4,4,4	4,4,4,4,4	1
G34	2543	1***	0***	4,4,4,4,4	4,4,4,4,4	1
G35	2544	0***	0***	4,4,4,4,4	4,4,4,4,4	1
G55	ATCC 11137	3***	0***	4,4,4,4,4	4,4,4,4,4	1
G56	ATCC 18133	17**	11***	4,4,4,4,4	3,4,4,4,4	1
G57	ATCC 26849	3***	0***	4,4,4,4,4	4,4,4,4,4	1
G63	0-1151	0***	0***	4,4,4,4,4	4,4,4,4,4	1
G64	0-1152	3***	1***	4,4,4,4,4	4,4,4,4,4	1
G67	0-0700	0***	0***	4,4,4,4,4	4,4,4,4,4	1
G70	0-0708	6***	1***	4,4,4,4,4	4,4,4,4,4	1
G71	0-0715	54*	73**	3,3,3,3,4	2,3,3,3,3	1
G73	0-0719	1***	0***	4,4,4,4,4	4,4,4,4,4	1
G74	0-0725	0***	0***	4,4,4,4,4	4,4,4,4,4	1
G75		7***	0***	4,4,4,4,4	4,4,4,4,4	1
G76		72*	3***	1,2,3,2,3	4,4,4,4,4	1
G28	FG 6-1	90	99	0,0,0,0,3	0,0,1,1,3	
G29	FG 6-2	104	76	0,0,0,0,0	0,0,0,1,4	
G30	FG 6-2H	107	110	0,0,0,0,2	0,0,0,0,0	
G58	ATCC 46082	99	90	0,0,0,0,0	0,0,0,1,3	
G65	0-0013	108	75	0,0,0,0,0	0,1,1,2,2	
G66	0-0699	107	101	0,0,0,0,0	0,0,0,0,1	
G68	0-0702	108	90	0,0,0,0,0	0,0,1,1,2	
G69	0-0705	104	96	0,0,0,0,0	0,0,0,0,1	
G72	0-0717	110	99	0,0,0,0,0	0,0,0,1,1	

^a In percentages of control values.

PP, cultivar Peter Pears; N, cultivar Nymph; Significance levels: * $P < 0.1$, ** $P < 0.01$ and *** $P < 0.001$.

ship between isolates, are shown in a dendrogram (Fig. 2, later). The RFLP groups corresponded with the observed vegetative compatibility groups.

RFLP group I is the most common group within our collection and contains isolates from widely separated geographic origins. All race 1 and race 2 isolates that belong to VCG 0340 are present in this group. The isolates within this group were clustered with a similarity of 78% or higher. Examples of banding patterns found within this group are shown in Fig. 1 (lanes 1 to 3). Hybridization patterns of two pathogenic, self-incompatible isolates from the ATCC (G55 and G56) are clustered in the same group.

RFLP group II corresponds to VCG 0341 isolates and was clustered with a least similarity of 78%. All isolates in this group belonged to race 2. The pattern of this second group (Fig. 1, lanes 4 and 5) was quite distinct from the first, only sharing the 3.5 and 2.3 kb *Hind*III fragments.

The two isolates obtained from crocus (Cr1 and Cr2), which were previously identified as race 2 and VCG 0342, could be related with an 89% similarity (RFLP group III). Only the fragment with a size of 2.8 kb linked this group to RFLP group I, but none of the fragments corresponded to RFLP group II. The RFLP banding pattern of Cr2 is shown in Fig. 1 (lane 6).

The remaining pathogenic isolates G25 (race 2)

Fig. 1. Hybridization patterns of *Hind*III-digested total DNA of *Fusarium oxysporum* f.sp. *gladioli* hybridized with the labelled probe D4. Lanes represent isolates from restriction fragment length polymorphism groups I to V. Numbers on the left refer to the size of marker fragments in kilobases (kb).

DISCUSSION

The 3400 bp probe generated from total DNA of *F. oxysporum* f.sp. *dianthi* (Manicom *et al.*, 1987) proved to be a useful tool for examination of the genetic variation in *F. oxysporum* f.sp. *gladioli*. Similar results have been published for *F. oxysporum* f.sp. *dianthi* with the use of this probe (Manicom *et al.*, 1990). Part of this probe has been studied in detail but if it represents part of a functional gene, this is still unknown (Manicom *et al.*, 1990). Irrespective of function, the probe allowed the distinction of three major RFLP groups among pathogenic isolates of *Fusarium oxysporum* f.sp. *gladioli*; these groups corresponded with vegetative compatibility groups. In addition, two self-incompatible isolates (G55 and G56) were shown to be associated with isolates from VCG 0340 based on their RFLP pattern, while another self-incompatible pathogenic isolate (G25) was not associated with isolates from any vegetative compatibility groups so far described. We also observed the hybridization of this probe to DNA from many *F. oxysporum* isolates that were not pathogenic to gladiolus. Manicom *et al.* (1987, 1990) observed that vegetative compatibility groups within *F. oxysporum* f.sp. *dianthi* could be discriminated with this probe, and that homology exists with DNA from *F. oxysporum* f.sp. *lycopersici*. These results and our data indicate that the probe (or at least part of it) hybridizes to a more or less repetitive, conserved sequence present in the genomes of various special forms of *F. oxysporum*.

RFLP dendrograms, as presented here, depend on the probe used and, partially, on the method of cluster analysis. Kistler *et al.* (1991) noted comparable, but not identical clusters after analysis of the hybridization patterns of genomic DNA revealed by Southern hybridization with different genomic clones. In our study, various different methods for analysing similarity and linkage were tested, but all yielded largely the same clusters (results not shown). From observation of hybridization of only one probe to DNA fragments, resulting from digestion with one restriction enzyme (other restriction enzymes resulted in banding patterns with fewer bands, data not shown), there is a complete correlation between RFLP groups with vegetative compatibility groups (Table 1). However, the clustering limits were chosen arbitrarily, but conservatively because we were less interested in differences between individual isolates than in genetic variation within the special form.

and G76 (possibly a distinct race) both had unique restriction fragment patterns (Fig. 1, lane 7, RFLP IV and lane 8, RFLP V). Although these isolates had some bands in common with RFLP groups I and II, the banding patterns were not closely linked to those of other groups (Fig. 2). Neither isolate could be assigned to a vegetative compatibility group (moreover, G25 is self-incompatible). Because these isolates are monotypic, they cannot be named groups.

Non-pathogenic strains had distinctive restriction fragment patterns (monotypic RFLP VI-XVI) that could only be linked with a similarity lower than 40%. The clustering analysis for several non-pathogenic isolates (G10, G26, G28, G58 and G69) is shown in Fig. 2.

The isolates G19 (race 1, VCG 0340) and G22 (non-pathogenic) exhibited only weak hybridization even after several attempts. Other isolates (G65, G66 and G30) failed to hybridize completely. These isolates were not included in further analysis. Two isolates identified as *F. proliferatum* (G13 and M685) yielded only one band with an approximate size of 2.8 kb (data not shown).

Fig. 2. Dendrogram of restriction fragment length polymorphisms of isolates of *Fusarium oxysporum* f.sp. *gladioli* based on Sørensen similarity and average linkage.

With the exception of G76, all pathogenic isolates from different geographic origin were identified as race 1, VCG 0340 and RFLP group I. These isolates probably originate from one source and have been distributed with the corns. VCGs 0341 and 0342 (RFLP groups II and III, respectively), comprising isolates of race 2 (Roebroek & Mes, 1992), were not found outside of the Netherlands. This can probably be explained by the fact that all foreign isolates were obtained from 'large-flowered' *gladiolus* cultivars, which are susceptible only to race 1.

In general, mtDNA RFLP patterns of isolates of *Fusarium oxysporum* are similar or identical within a vegetative compatibility group, but quite different between vegetative compatibility groups (Kistler *et al.*, 1987; Jacobson & Gordon, 1990; Correll, 1991). This is true even when different races exist within any vegetative compatibility group. Within *F. oxysporum* from crucifers (Kistler *et al.*, 1987), each special form consisted

of only one vegetative compatibility group, and therefore a common ancestry for distinct races within one of these special forms from crucifers has been hypothesized. In studies where numerous vegetative compatibility groups were identified within a special form, it has been suggested that a special form is a collection of genetically diverse groups, possibly polyphyletic in origin (Jacobson & Gordon, 1990; 1991; Elias *et al.*, 1990). From the little homology we have found between vegetative compatibility groups (Fig. 2) we conclude that the special form *gladioli* probably also has a polyphyletic origin.

We suggest that VCG/RFLP groups are natural assemblages within f.sp. *gladioli* and that each has gained pathogenicity independently. Therefore, the mechanistic basis for pathogenicity of races from different VCG/RFLP groups may differ. Further mutations affecting pathogenicity could occur within a vegetative compatibility group and result in the occurrence of

different races. Correll (1991) has described such a model of race-VCG diversity in *Fusarium oxysporum*. *Fusarium oxysporum* f.sp. *gladioli* race 2 isolates from different VCG/RFLP groups are unified solely by their common pathogenicity to two differential hosts. Those differential cultivars were chosen as representatives of larger groups. Among gladioli, no other cultivars have been found that allow further differentiation of isolates (results not shown). Preliminary experiments have revealed, however, that there may be additional differential genotypes within other Iridaceae. It is questionable whether different vegetative compatibility groups, pathogenic to a different set of host plant genera, species or cultivars within the Iridaceae, should be grouped within one single special form. Further analysis of host plant species susceptibility may support a division into more than one special form or suggest a simplified relationship between races and VCG/RFLP group.

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REFERENCES

- Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC, 1987. Intraspecific phylogeography: mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecological Systematics* **18**, 489–522.
- Bald JG, Suzuki T, Doyle A, 1971. Pathogenicity of *Fusarium oxysporum* to Easter lily, *Narcissus* and *Gladiolus*. *Annals of Applied Biology* **67**, 331–42.
- Beckman JS, Soller M, 1983. Restriction fragment length polymorphisms in genetic improvement: methodologies, mapping and costs. *Theoretical and Applied Genetics* **67**, 35–43.
- Boerema GH, Hamers MEC, 1989. Check-list for scientific names of common parasitic fungi. Series 3b: Fungi on bulbs: Amaryllidaceae and Iridaceae. *Netherlands Journal of Plant Pathology* **95** (Suppl. 3), 1–32.
- Coddington A, Matthew PM, Cullis C, Smith KA, 1987. Restriction digest patterns of total DNA from different races of *Fusarium oxysporum* f.sp. *pisi*—an improved method for race classification. *Journal of Phytopathology* **118**, 9–20.
- Correll JC, 1991. The relationship between formae speciales, races, and vegetative compatibility groups in *Fusarium oxysporum*. *Phytopathology* **81**, 1061–4.
- Digby PGN, 1984a. Dendrograms and ziggurats. *Genstat Newsletter* **14**, 14–18.
- Digby PGN, 1984b. Drawing pretty dendrograms. *Genstat Newsletter* **14**, 18–26.
- Elias KS, Katan T, Zamir D, 1990. RFLP based phylogeny of *Fusarium oxysporum* f.sp. *lycopersici* reveals no association between race and genetically isolated populations. *Phytopathology* **80**, 980.
- Jacobson DJ, Gordon TR, 1988. Vegetative compatibility and self-incompatibility within *Fusarium oxysporum* f.sp. *melonis*. *Phytopathology* **78**, 668–72.
- Jacobson DJ, Gordon TR, 1990. Variability of mitochondrial DNA as an indicator of relationships between populations of *Fusarium oxysporum* f.sp. *melonis*. *Mycological Research* **94**, 734–44.
- Jacobson DJ, Gordon TR, 1991. *Fusarium oxysporum* f.sp. *melonis*: a case study of diversity within a forma specialis. *Phytopathology* **81**, 1064–7.
- Kistler HC, Bosland PW, Benny U, Leong S, Williams PH, 1987. Relatedness of strains of *Fusarium oxysporum* from crucifers measured by examination of mitochondrial and ribosomal DNA. *Phytopathology* **77**, 1289–93.
- Kistler HC, Momol EA, Benny U, 1991. Repetitive genomic sequences for determining relatedness among strains of *Fusarium oxysporum*. *Phytopathology* **81**, 331–6.
- McClellan WD, 1945. Pathogenicity of the vascular *Fusarium* of gladiolus to some additional iridaceous plants. *Phytopathology* **35**, 921–30.
- Maniatis T, Fritsch EF, Sambrook J, 1982. *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press.
- Manicom BQ, Bar-Joseph M, Rosner A, Vigodsky-Haas H, Kotze JM, 1987. Potential application of random DNA probes and restriction fragment length polymorphisms in the taxonomy of the Fusaria. *Phytopathology* **77**, 669–72.
- Manicom BQ, Bar-Joseph M, Kotze JM, Becker MM, 1990. A restriction fragment length polymorphism probe relating vegetative compatibility groups and pathogenicity in *Fusarium oxysporum* f.sp. *dianthi*. *Phytopathology* **80**, 336–9.
- Massey LM, 1926. *Fusarium* rot of gladiolus corms. *Phytopathology* **16**, 509–23.
- Michelmore RW, Hulbert SH, 1987. Molecular markers for genetic analysis of phytopathogenic fungi. *Annual Review Phytopathology* **25**, 383–404.
- Nelson PE, Horst RK, Woltz SS, 1981. *Fusarium* diseases of ornamental plants. In: Nelson PE, Tousoun TA, Cook RJ, eds. *Fusarium: Disease, Biology and Taxonomy*. Philadelphia, PA, USA: Pennsylvania State University Press, 121–8.
- Payne RW, Lane PW, Ainsley AE, Bicknell KF, Digby PGN, Harding SA, Leech PK, Simpson HR, Todd AD, Verrier PJ, White RP, Gower JC, Tunnicliffe Wilson G, Paterson LJ, 1987. *GENSTAT 5 Reference Manual*. Oxford, UK: Clarendon Press.

- Puhalla JE, 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Canadian Journal of Botany* **63**, 179–83.
- Puhalla JE, Spieth PT, 1985. A comparison of heterokaryosis and vegetative incompatibility among varieties of *Gibberella fujikuroi* (*Fusarium moniliforme*). *Experimental Mycology* **9**, 39–47.
- Roebroeck EJA, Groen NPA, Mes JJ, 1990. Detection of latent *Fusarium oxysporum* in gladiolus corms. *Acta Horticulturae* **266**, 469–76.
- Roebroeck EJA, Mes JJ, 1992. Physiological races and vegetative compatibility groups within *Fusarium oxysporum* f.sp. *gladioli*. *Netherlands Journal of Plant Pathology* **98**, 57–64.

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