Theor Appl Genet (1990) 79:641–645

Genetic analysis of resistances to races 1 and 2 of *Fusarium oxysporum* f. sp. *lycopersici* from the wild tomato *Lycopersicon pennellii* *

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Received December 20, 1989; Accepted January 22, 1990
Communicated by F. Salamini

Summary. Resistance to race 3 of Fusarium wilt in the wild tomato *Lycopersicon pennellii* (LA 716) was previously found to be controlled by one major locus, *I-3*, tightly linked to *Got-2* on chromosome 7. This accession was also found to carry resistance to races 1 and 2; a genetic analysis of these resistances is reported in this paper. This analysis proceeded in two steps. First, allelism tests demonstrated that race 1 and 2 resistances carried by *L. pennellii* were not allelic to the *I* and *I-2* genes originally incorporated into *L. esculentum* from *L. pimpinellifolium*. Second, an interspecific backcross with *L. pennellii* (BC_1) was used to determine the mode of inheritance of these new resistances and their chromosomal location by segregation and linkage analysis. BC_1 responses to each of the races were determined using progeny tests (BC_1S_1). BC_1S_1 plants were inoculated with race 1 or 2 and evaluated after 1 month using a visual disease rating system; mean disease ratings were calculated for each BC_1 individual for each race based on the progeny scores. A bimodal frequency distribution of the BC_1 mean disease ratings was observed for both races, indicating that one major locus controlled resistance in each case. Statistical comparisons of the mean disease ratings of homozygous versus heterozygous individuals at each of 17 segregating enzyme loci were used to map the resistances to races 1 and 2. Tight linkage was detected between the enzyme locus *Got-2* and resistances to both races, as was previously reported for the *I-3* locus. Therefore, the *Got-2* locus can be used as a selectable marker for resistances to all three races. The relationship of these resistances is discussed in the paper. In addition, as previously reported for race 3, significance was also detected for the chromosome segment marked by *Aps-2* on chromosome 8 for both races. Currently many cultivars carry *I* and *I-2* resistances to races 1 and 2. Incorporation of the LA 716 resistances to these two races into cultivars may reduce the likelihood of new race development.

Key words: Fusarium wilt – Tomato – Disease resistance – Isozyme – Linkage

Introduction

Race 3 of the tomato Fusarium wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen is spreading throughout important growing regions worldwide. It has been reported in Australia (Grattidge and O'Brien 1982), Florida (Volin and Jones 1982), and California (Davis et al. 1988). A dominant gene, *I-3*, that controls for resistance to race 3 has been detected in the wild tomato species *Lycopersicon pennellii* (Corr.) D'Arcy, accession LA 716 (Scott and Jones 1989), and found to be tightly linked (2.5 cM) to the *Got-2* locus on chromosome 7 (Bournival et al. 1989). Since LA 716 was also found to be resistant to races 1 and 2 (Scott and Jones 1989), a genetic analysis was conducted to elucidate the relationships among the resistances to the three races.

Two parallel approaches were taken for this analysis. First, although many commercial cultivars already carry resistances to races 1 and 2 (*I* and *I-2* on chromosome 11) derived for *L. pimpinellifolium* accessions (Bohn and Tucker 1939, 1940; Paddock 1950; Alexander and Hoover 1955; Stall and Walter 1965; Latterot 1976), it was of interest to determine whether LA 716 carried ad-

* Florida Agricultural Experiment Station, Journal Series No. R-00205
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ditional genes for resistance to these two races. For this reason, allelism of *I* and *I-2* with those resistances present in LA 716 was tested. In the second approach, the chromosomal locations of genes controlling for resistances to races 1 and 2 found in LA 716 were determined using linkage analyses to 17 previously mapped enzyme loci.

Materials and methods

Plant material

Two different progenies were generated for the allelism test using the *L. esculentum* cultivar Hayslip; this cultivar carries *I* and *I-2*. The first progeny was an F_2 of the cross 'Hayslip' × LA 716. Detection of susceptible individuals in this progeny would indicate that the genes were nonallelic. A second progeny [Bonny Best × (Hayslip × LA 716)] was also analyzed because of the increased chances of observing susceptible plants in a nonallelic interaction.

The interspecific backcross, *L. esculentum* × (*L. esculentum* × *L. pennellii*, LA 716), analyzed previously for the genetics of race 3 resistance (Bournival et al. 1989), was used in this study to analyze the responses to races 1 and 2 through progeny (BC_1S_1) tests. 'Bonny Best' was used as the *L. esculentum* parent because of its extreme susceptibility to all three races. The 54 BC_1 individuals used in this analysis (those for which BC_1S_1 seed was still available) were a subset of the 79 individuals previously used to locate *I-3* (Bournival et al. 1989). Chi-square analyses indicated that, for all marker loci, ratios of homozygotes to heterozygotes in the subset did not differ significantly from those observed in the overall population. In addition, a *Z*-test indicated that the race 3 mean disease rating of the subset was not different from that of the overall population.

Isozyme analysis and inoculations

Isozyme analysis was conducted on the BC_1 individuals as described previously (Bournival et al. 1989). Backcross individuals were scored for each of 17 enzyme loci including *Prx-1, Skdh-1, Bnag-1,* and *Dia-2* on chromosome 1; *Est-7* and *Prx-2* on chromosome 2; *Prx-7* on chromosome 3; *Pgm-2* on chromosome 4; *Aps-1* on chromosome 6; *Got-2* on chromosome 7; *Aps-2* on chromosome 8; *Prx-4* on chromosome 10; *Sod-1* on chromosome 11; and *Est-4, 6Pgdh-2, Pgi-1,* and *Aco-1* on chromosome 12. BC_1 individuals were self-pollinated to produce BC_1S_1 seed for progeny tests.

BC_1S_1 individuals and the progenies used in the allelism test were inoculated according to previously described procedures (Bournival et al. 1989) with races 1 and 2 of *F. oxysporum* f. sp. *lycopersici* (race 1: strain SC626, isolated by Dr. M. Cirulli in Italy, also called the 'Oristano' isolate; race 2: strain SC548, isolated by Dr. J. M. Walter at the University of Florida at Bradenton). Included in each set of inoculations were 'Bonny Best,' LA 716, 'Manapal' (resistant to race 1 only), 'Hayslip,' and the F_1 (Bonny Best × LA 716) to monitor the activity and specificity of inoculum. In addition, another set of controls was treated with distilled water only. Plants were evaluated 10–14 days after inoculation and again approximately 1 month after inoculation using the visual rating system of Bournival et al. (1989). A mean disease rating was calculated for each backcross individual for each race using the progeny scores; at least 20 progeny were used for each analysis. All statistical analyses were performed on a mainframe computer using the Statistical Analysis System (SAS Institute Inc., SAS Circle, Box 8000, Cary/NC).

Results

The detection of susceptible plants in F_2 progenies inoculated with race 1 or 2 indicated that neither *I* or *I-2* were allelic to the resistances carried by LA 716 (Table 1). The same conclusion was more decisively reached with the results of the *L. esculentum* backcross progeny, where several susceptible plants were observed for each race: for race 1, 39.6% and for race 2, 39.4% (Table 1).

Frequency distributions of BC_1 mean disease ratings for races 1 and 2 were found to be bimodal according to the coefficient of bimodality ($b=(m_3^2+1)/(m_4+3)$, m_3 = skewness, m_4 = kurtosis; race 1: $b=0.563$; race 2: $b=0.584$; $\alpha<0.05$). A bimodal distribution was also observed for the race 3 mean disease ratings (Bournival et al. 1989). These results indicated that a single major locus was responsible for resistances to races 1 and 2, as was the case for race 3.

The possible association of resistance genes with any of the 17 segregating enzyme markers was tested by comparing the mean disease ratings of homozygotes and heterozygotes at each marker locus; a one-tailed nonparametric analysis of variance was used for these tests as previously described (Bournival et al. 1989). Heterozygotes for the chromosome segment marked by *Got 2* were markedly more resistant than homozygotes for both races 1 and 2 (Table 2). Since it was recently reported that *I-3*, a gene conferring race 3 resistance, was also tightly linked to *Got-2* (Bournival et al. 1989), these results indicated that resistances to the three races were either controlled by *I-3* alone or by gene(s) tightly linked to *I-3*. Moreover, as previously reported for race 3 (Bournival et al. 1989), significant effects were also observed for the chromosome segment marked by *Aps-2* for both races (Table 2) suggesting that *Tfw*, a factor linked to *Aps-2*, was active against all three races.

The chromosome segment marked by *Prx-4* also appeared to have an effect for both races. However, this was determined to be an artifact, since a two-way contingency table comparing *Got-2* and *Prx-4* showed slight skewing ($\alpha<0.05$) in favor of parental types (66%). This resulted in more BC_1 individuals that were homozygous or heterozygous for both loci than expected. Since the chromosome segment marked by *Got-2* was significant for both races (Table 2), the significance observed for *Prx-4* can be attributed to skewing with the resistance gene(s) linked to *Got-2*. In addition, significance for the *Prx-4* marker was not observed in the original population ($N=79$) analyzed for race 3 resistance (Bournival et al. 1989). Other marker loci assorted as previously reported (Tanksley and Rick 1980; Bournival et al. 1988; Chetelat 1989) with no significant skewing being observed between unlinked loci.

In order to discern whether *I-3* alone or other tightly linked loci were responsible for resistances to races 1 and

Table 1. Results of the inoculations for allelism tests for *I* and *I-2* resistances with LA 716 resistances to races 1 and 2. Visual ratings were given 1 month after inoculations

Cross[b]	Race	Visual rating (no. of plants)[a]					Total no. plants	% susceptible[c]
		1	2	3	4	5		
Controls								
Bonny Best	1	0	0	0	0	29	29	100.0
	2	0	0	0	0	34	34	100.0
LA 716	1	21	0	0	0	0	21	0.0
	2	14	0	0	0	0	14	0.0
Bonny Best × LA 716	1	25	5	0	0	0	30	0.0
	2	39	3	0	0	0	42	0.0
Manapal	1	17	8	1	1	0	27	7.4
	2	1	1	7	14	28	51	96.1
Hayslip	1	15	5	1	0	0	21	4.8
	2	13	7	2	1	0	23	13.0
Allelism progenies								
F_2 of Hayslip × LA 716	1	32	1	0	0	1	34	2.9
	2	32	2	0	0	1	35	2.8
BB × (Hayslip × LA 716)	1	31	4	9	8	6	58	39.6
	2	48	9	7	6	24	94	39.4

[a] Denotes the number of plants falling into each visual rating category; 1, resistant to 5, susceptible
[b] BB Bonny Best: *i/i, i-2/i-2;* Manapal: *I/I, i-2/i-2;* Hayslip: *I/I, I-2/I-2*
[c] Plants with a visual rating of 3–5 were classified as susceptible whereas ratings of 1 and 2 were considered resistant

Table 2. Comparisons of BC_1 mean disease ratings using a one-way nonparametric analysis of variance – the Wilcoxon rank sum test. In this procedure, BC_1 individuals were ranked according to their mean disease rating, and homozygote and heterozygote mean disease rankings were compared at each marker locus for each race. ee – *esculentum* homozygotes; ep – *pennellii* heterozygotes; R1, R2, and R3 – races 1, 2, and 3, respectively; NS – not significant[a]

Locus	Chromosome no.	No. plants		R1 mean disease ranking		R2 mean disease ranking		R3 mean disease ranking		Wilcoxon rank sum test (*Z*-statistic)		
		ee	ep	ee	ep	ee	ep	ee	ep	R1	R2	R3
Prx-1	1	16	38	26.5	27.9	28.3	27.7	(26.4	28.0)	NS	NS	(NS)
Skdh-1	1	16	35	27.5	25.3	28.7	24.8	(28.2	25.0)	NS	NS	(NS)
Bnag-1	1	14	29	22.0	22.0	23.2	21.4	(22.6	21.7)	NS	NS	(NS)
Dia-2	1	12	35	24.7	23.8	28.2	22.5	(28.1	22.6)	NS	NS	(NS)
Est-7	2	30	24	27.2	27.8	28.2	26.7	(27.8	27.1)	NS	NS	(NS)
Prx-2	2	25	23	23.3	25.8	23.5	25.6	(22.6	26.5)	NS	NS	(NS)
Prx-7	3	24	25	25.4	24.6	23.0	26.9	(23.3	26.6)	NS	NS	(NS)
Pgm-2	4	26	28	24.0	30.7	25.5	29.4	(24.2	30.5)	NS	NS	(NS)
Aps-1	6	28	23	25.9	26.2	26.6	25.2	(25.3	26.8)	NS	NS	(NS)
Got-2	7	29	25	39.6	13.5	40.0	13.0	(40.0	13.0)	−6.1**	−6.3**	(−6.3**)
Aps-2	8	31	23	31.4	22.2	31.6	22.0	(31.6	22.0)	−2.1*	−2.2*	(−2.2*)
Prx-4	10	30	23	32.1	20.4	30.2	22.8	(31.2	21.6)	−2.7*	−1.7*	(−2.2*)[b]
Sod-1	11	18	19	20.6	17.5	20.0	18.1	(20.7	17.4)	NS	NS	(NS)
Est-4	12	15	25	23.9	18.5	24.6	18.1	(22.9	19.1)	NS	NS	(NS)
6Pgdh-2	12	15	37	26.5	26.5	26.7	26.4	(25.8	26.8)	NS	NS	(NS)
Pgi-1	12	14	40	26.8	27.8	26.8	27.8	(25.5	28.2)	NS	NS	(NS)
Aco-1	12	20	34	25.2	28.8	23.5	29.8	(24.5	29.3)	NS	NS	(NS)

[a] Race 3 mean disease rankings and *Z*-statistics calculated using only the 54 individuals analyzed for races 1 and 2
[b] In the original population ($N=79$) anlayzed for race 3, the chromosome segment marked by *Prx-4* was not significant (Bournival et al. 1989)
*,** Significant at 0.05 and 0.0001 levels, respectively

Table 3. Mean disease ratings and cluster classifications of putative recombinants between resistances to races 1 and 2/3. BC_1 individuals were classified as resistant or susceptible for each race using the average linkage clustering method based on their mean disease ratings. R1, R2, and R3 – races 1, 2, and 3, respectively; R and S – resistant and susceptible[a]

BC #	Cluster classification			Mean disease rating		
	R1	R2	R3	R1	R2	R3
12	R	S	S	3.15	3.78	3.49
22	R	S	S	3.29	3.46	3.64
41	R	S	S	3.64	4.08	3.92
65	R	S	S	2.90	3.30	3.05
71	R	S	S	3.59	4.00	3.96
92	R	S	S	3.36	3.77	3.06
130	R	S	S	3.24	3.63	3.70
Ranges						
Resistant cluster				1.55–3.64	1.39–2.86	1.50–2.70
Susceptible cluster				3.78–4.88	3.30–4.93	3.05–4.97

[a] Data for race 3 clustering analysis was obtained from Bournival et al. (1989)

2, the genotype (resistant or susceptible) at each of the putative race-specific loci was deduced for all BC_1 individuals based on mean disease ratings of their progeny. For this purpose, the average linkage clustering analysis was used as described previously (Bournival et al. 1989). Two-way contingency tables were used to test for independent assortment between the putative loci that control resistances to the three races. No recombinants were detected between resistances to races 2 and 3; however, seven BC_1 individuals appeared to be recombinants between race 1 resistance and the other two resistances (Table 3).

Discussion

The data presented here seem to indicate that *I-3* confers resistance to races 2 and 3, and that another linked gene on chromosome 7 controls for resistance to race 1. However, a closer examination of the seven putative recombinants between race 1 resistance and resistances to races 2 and 3 suggests that they may have been misclassified by the cluster analysis. First, mean disease ratings of the putative recombinants were very close to the cutoff point for the resistant class for race 1 (Table 3). In addition, all seven recombinants appeared in the same class – resistant for race 1 and susceptible for races 2 and 3 (Table 3). This was due to the fact that the upper limit of the race 1 resistant cluster was much greater than the limits for races 2 and 3 (Table 3). The presence of minor factors, such as *Tfw*, increases the frequency of individuals with intermediate disease ratings; these individuals have a greater probability of being misclassified. An additional reason for the discrepancy in clustering could have been due to the slightly greater aggressiveness of race 1 compared to the other two races. For instance, all F_1 (Bonny Best × LA 716) plants evaluated were given a rating of 1 or 2 for all three races (Table 1). However, a 2 rating was given to 16.7% of the F_1s analyzed for race 1 compared to only 7.1% and 4.1% for races 2 and 3, respectively. Though no clear recombinants were observed between race resistances, the possibility of more than one resistance gene on chromosome 7 could not be ruled out with the available data.

In the past, the Fusarium wilt pathogen has been able to overcome host genes for resistance (*I* and *I-2*) and infect commercial tomato cultivars. Races 1 and 2 have spread to most tomato-growing regions, and still pose the threat of forming a new race. Since *Got-2* is tightly linked to monogenic resistances to all three races, it can be used as a selectable marker to incorporate genetic resistances to each of the races into new cultivars. This might reduce the probability of a new race forming in the near future. In addition, if *Tfw* were tagged with a chromosome 8 marker, its incorporation into new cultivars might offer even more stable resistance to each of the races. It should be noted that only one isolate of each of the races was used as a source of inoculum in this study and, therefore, these results should be verified with other isolates.

Previous reports have concluded that genes for resistance to obligate parasites such as *Puccinia, Uromyces,* and *Erysiphe* are the product of coevolution between these pathogens and their hosts (Anikster and Wahl 1979; Eshed and Dinoor 1981; Moseman et al. 1983, 1984). *F. oxysporum* is a facultative parasite with the ability to survive away from its host. An interesting feature of the *L. pennellii* resistance to Fusarium wilt is that it may not be the result of selection pressure on the host. The natural habitat of *L. pennellii* (LA 716) is in the deserts of southern Peru, where it grows on the slopes of rocky soils with nearly no precipitation (Rick and Tanksley 1981). Either the tomato Fusarium wilt pathogen can adapt to the extreme environment of *L. pennellii,* or the resistance to the fungus is not the result of coevolution between pathogen and host. A survey of Fusarium populations and an assessment of their pathogenic activity in the area of distribution of *L. pennellii* could elucidate whether LA 716 may have had an evolutionary interaction with *F. oxysporum.*

Acknowledgements. This research was supported in part by a grant from the Florida Tomato Exchange. We would like to thank Dr. D. J. Cantliffe for making his growth chambers available for this study and Dr. J. P. Jones for providing the Fusarium isolates. We are also appreciative of the critical reviews and valuable suggestions of Dr. R. E. Stall and Dr. H. C. Kistler.

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