New Sources of Genetic Resistance to Race 3 of Fusarium Wilt of Tomato

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ABSTRACT

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Wild *Lycopersicon* accessions were screened for resistance to race 3 of *Fusarium oxysporum* f. sp. *lycopersici*. Of the accessions examined, only *L. hirsutum* (LA 1777) and *L. parviflorum* (LA 2133) consistently failed to develop wilt symptoms after inoculation. Resistances in these two accessions were analyzed genetically. Results from these analyses suggested that the resistances were not monogenic dominant or monogenic recessive and, therefore, genetically different from the resistance observed previously in *L. pennellii* (LA 716). Resistance in *L. parviflorum* (LA 2133) could be controlled by two independent recessive genes. We propose that genes involved in the resistances expressed in *L. hirsutum* (LA 1777) and *L. parviflorum* (LA 2133) be tagged with molecular markers and incorporated along with resistance genes derived from *L. pennellii* (LA 716) into commercial cultivars. This stacking of resistance genes could delay the appearance of new races of economic importance.

Breeding for resistance to a disease involves three basic steps: screening potential sources of resistance, analyzing the inheritance of resistance in promising genotypes, and incorporating the resistance gene(s) into new cultivars. Breeders have traditionally chosen to work with qualitatively inherited resistances because they are easier to transfer into commercial cultivars. Unfortunately, pathogens are usually able to overcome qualitative resistances and again successfully cause disease in the host. Breeders have been reluctant to work with quantitatively inherited resistance because of its polygenic nature and the subsequent need to screen very large populations. This problem is aggravated by environmental interactions, resulting in slow progress toward the development of new cultivars. However, it is now possible to identify and easily transfer loci affecting quantitative traits through the use of linked marker isozyme and restriction fragment length polymorphism (RFLP) loci (9,12,13).

The causal agent of Fusarium wilt of tomato, *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hansen, has overcome monogenic resistances to races 1 and 2, controlled by the *I* and *I-2* genes, respectively (1,2,4,8,14). Monogenic resistance to race 3 (*I-3*) has been detected in *Lycopersicon pennellii* (Cor.) D'Arcy (LA 716) (11); this gene is currently being incorporated into new cultivars through selection by using a tightly linked isozyme locus, *Got-2* on chromosome 7 (5). In addition, a minor resistance factor designated *Tfw* for "tolerance to Fusarium wilt," linked to *Aps-2* on chromosome 8, was also found in LA 716 (5). These findings prompted us to survey other *Lycopersicon* accessions for additional sources of resistance to race 3 of *F. oxysporum* f. sp. *lycopersici*. A wide variety of wild

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Lycopersicon species were screened for resistance to race 3, and resistant genotypes were genetically analyzed with F_2 and BC_1 progenies derived from crosses to *L. esculentum*.

MATERIALS AND METHODS

Plant material. One to three accessions of six *Lycopersicon* species from a very wide range of habitats were selected for the screening (Table 1). Seeds of selected accessions were originally obtained from the Tomato Genetics Cooperative collection, courtesy of C. M. Rick, University of California at Davis, and increased at the University of Florida by controlled pollinations. The collection sites of these accessions extended from the Galapagos Islands in Ecuador to the southern tip of Peru. The climates ranged from temperate with year-round rainfall to a desert with almost no precipitation, and elevations ranged from sea level to 3,200 m (Table 1). The accessions included: *L. esculentum* var. *cerasiforme* (Dun.) A. Gray (LA 2121 and LA 1310); *L. pimpinellifolium* (Jusl.) Mill. (LA 2097, LA 1582, and LA 1670); *L. cheesmanii* Riley (LA 1449); *L. chmielewskii* Rick (LA 1306); *L. parviflorum* Rick (LA 2133); *L. hirsutum* Humb. & Bonpl. (LA 1777 and LA 2409); and *L. hirsutum* Humb. & Bonpl. var. *glabratum* Muller (LA 1625) (Table 1). All accessions were cross-compatible with *L. esculentum*. Backcross and F_2 progenies were generated between the extremely susceptible *L. esculentum* 'Bonny Best' and each race 3 resistant accession for the genetic analysis; Bonny Best was used as the pistillate parent in all crosses.

Inoculations. Inoculation procedures were those described previously (5). Inoculum of *F. o.* f. sp. *lycopersici*, race 3, isolate SC761, was obtained from J. P. Jones at the University of Florida, Gulf Coast Research and Education Center. Two sets of inoculations, including all accessions, were conducted. The spore concentration was 2.5×10^7 spores per milliliter. Plants were monitored for external signs of wilting and stunting. Included in these inoculations were Bonny Best and *L. pennellii* (LA 716) plants as susceptible and resistant controls, respectively (5,11). A set of all accessions including Bonny Best and LA 716, were treated with distilled water as additional controls.

Those accessions that showed no external wilt symptoms in both inoculations were further analyzed for the inheritance of resistance. For this analysis, F_2 and BC_1 progenies derived from crosses to Bonny Best were inoculated and evaluated according to the visual rating system of Bournival et al (5). In this system, plants are rated from 1 (completely resistant) to 5 (susceptible with external wilt symptoms); ratings 2–4 are intermediate phenotypes based on the level of vascular browning observed. In addition, a control set including Bonny Best, LA 716, and their F_1, BC_1, and F_2 progenies was inoculated for the purpose of comparing segregation of resistance in LA 716 with those observed in the new accessions. The spore concentration for these inoculations was $1.10 - 1.25 \times 10^8$ spores per milliliter. Distilled water treatments of Bonny Best, LA 716, their F_1, and the accession(s) being tested were also included as additional controls.

RESULTS

Results of the two sets of inoculations of the *Lycopersicon* accessions were in general agreement and were thus pooled (Table 2). In both inoculations, all Bonny Best controls were infected, whereas none of the *L. pennellii* (LA 716) plants showed symptoms of wilt (Table 2). Most of the accessions had a lower proportion of wilted individuals compared with Bonny Best (Table 2). The greatest levels of resistance were detected in *L. hirsutum* (LA 1777) and *L. parviflorum* (LA 2133), both of which had no plants with external wilt symptoms (Table 2). These two accessions represent potential new sources of genes for resistance to race 3 of Fusarium wilt of tomato.

Resistances to race 3 in *L. hirsutum* (LA 1777) and *L. parviflorum* (LA 2133) were investigated independently by using a segregation analysis of backcross (BC_1) and F_2 progenies. The "F_2" progeny for the self-incompatible *L. hirsutum* (LA 1777) was obtained through mass sib pollinations involving five F_1 plants. Also, three separate BC_1 progenies were generated for this accession by using different F_1 plants; this was done to increase the chances of detecting qualitative resistance that may have been segregating in the highly heterogeneous LA 1777 population. F_2 and BC_1 progenies for *L. parviflorum* (LA 2133) were derived from a single F_1 individual.

Analysis of the *L. hirsutum* (LA 1777) progenies revealed that its race 3 resistance is not monogenic dominant. All inoculated F_1 individuals grew vigorously and showed a level of resistance greater than that usually observed in Bonny Best; however, most of the BC_1 individuals showed external wilt symptoms, and none could be categorized as resistant to race 3 (Table 3). The presence of resistant F_1 individuals suggests that resistance in LA 1777 is not completely under recessive control. However, it should be noted that the F_1 population analyzed was small, and the possibility of the resistant individuals being escapes could not be ruled out. Unfortunately, the limited size of the F_2 progeny caused by lack of seed availability makes it difficult to completely assess the complexity of resistance.

Resistance in *L. parviflorum* (LA 2133) is also not under monogenic dominant control; all of the 12 BC_1 individuals inoculated were completely

Table 1. *Lycopersicon* accessions screened for resistance to race 3 of *Fusarium oxysporum* f. sp. *lycopersici*

Species	Accession	Country[a]	Compatibility reaction[b]	Climate classification[c]	Mean annual temperature (C)	Mean annual rainfall (mm)	Elevation (m)
L. esculentum	LA 2121	Ec	SC	Cf	20	700	1,000
var. *cerasiforme*	LA1310	Pe	SC	Aw	22	2,000	2,700
L. pimpinellifolium	LA 2097	Ec	SC	Aw	17	650	700
	LA 1582	Pe	SI	Cw	22	750	0
	LA 1670	Pe	SC	BSs	16	<100	500
L. cheesmanii	LA 1449	Ec	SC	Bw	22	?	0
L. chmielewskii	LA 1306	Pe	SI	Cw	16	1,300	2,000
L. parviflorum	LA 2133	Ec	SC	Cw	17	650	2,000
L. hirsutum	LA 1777	Pe	SI	Dwb	10	300	3,200
	LA 2409	Pe	SC	Bw	18	<100	3,000
L. hirsutum var. *glabratum*	LA 1625	Ec	SC	Aw	>23	700	100
L. pennellii	LA 716	Pe	SC	Bw	18	<100	150

[a]Ec = Ecuador, Pe = Peru.
[b]SC = self-compatible, SI = self-incompatible.
[c]Climate classifications for accession collection sites according to the system of W. Koppen (7): Aw = tropical savanna climate, dry in winter; BSs = steppe climate, semiarid, rains in winter, dry in summer; Bw = desert climate, arid, driest in winter; Cf = temperate climate, rainfall all through the year; Cw = temperate climate, periodically dry periods, usually in the winter; Dwb = cold climate, humid and very cold, coldest month below 0 C, dry in the winter.

susceptible (Table 3). However, the F_2 progeny fits a 7:9 ratio (resistant:susceptible), suggesting that this resistance is controlled by two independent recessive genes (Table 4). Unfortunately, F_1 progeny could not be analyzed because of poor germination.

DISCUSSION

These studies indicate that *L. hirsutum* (LA 1777) and *L. parviflorum* (LA 2133) resistances to race 3 of *F. oxysporum* f. sp. *lycopersici* are not under monogenic control. However, it is difficult to determine the number of genes involved in the resistances based on the segregation ratios. Unlike *L. pennellii* (LA 716) and the F_1 (Bonny Best × LA 716), some of the inoculated parental LA 1777 and LA 2133 plants fell into disease rating classes 3 and 4, indicating that internal wilt symptoms are occasionally associated with these resistances. Also, it has been observed previously that in interspecific *Lycopersicon* crosses, monogenic traits occasionally show skewed ratios (5,10,12). In addition, if more than one gene is involved, not all genes would be expected contribute equally toward resistance. For instance, LA 716 BC_1 and F_2 progenies do not fit the expected ratios for monogenic dominant resistance; an excess of resistant types is observed in both progenies. The lack of fit to a monogenic model is due to the presence of at least one minor factor, *Tfw* (5). Although *Tfw* clearly confers resistance to Fusarium wilt, its contribution is not nearly as great as that of *I-3* (5). BC_1 progeny do fit the expected ratio for two dominant genes; however, because of an excess of susceptible individuals, F_2 progeny do not fit this model.

In the past, polygenic resistances were not considered useful to plant breeders. However, major genes involved in quantitative traits can now be tagged through linkage to loci of naturally occurring isozyme and DNA restriction fragments. This is possible in tomato, where an extensive RFLP/isozyme linkage map has been developed (3,6,13). Even though monogenic dominant resistance to race 3 (*I-3*) has been detected and currently is being incorporated into commercial cultivars, a race that can overcome the *I-3* resistance may appear in the future. New races might arise either through strong selection pressure on extant races present at very low levels, or through mutation of one of the dominant races. Incorporation of several non-allelic genes from different sources may limit the number of fungal genotypes capable of causing disease, thus restricting, or at the very least, delaying the development of a new race. Further characterization of the resistances detected in *L. hirsutum* (LA 1777) and *L. parviflorum* (LA 2133) followed by the tagging of the resistance genes with molecular markers (isozymes or DNA restriction fragments) will facilitate their incorporation into genotypes that already may carry *I-3* and *Tfw*.

It is interesting that such genetically distinct accessions as LA 716, LA 2133, and LA 1777 all possess resistance to the same strain of the tomato wilt pathogen. These accessions represent three different species and come from markedly different habitats. The natural habitat of *L. pennellii* (LA 716) is in southern Peru near sea level in a desert climate with nearly no precipitation, whereas that of *L. hirsutum* (LA 1777) is in central Peru at an elevation of 3,200 m in a cold climate with a mean temperature of 10 C. *L. parviflorum* (LA 2133) occupies a habitat in Ecuador at an elevation of 2,000 m with a temperate climate (7). One possible explanation is that resistance to *Fusarium* developed through mutation/selection before the divergence of *Lycopersicon* into present species and ecological niches. If this were the case, resistances in the accessions should be similar. However, *L. parviflorum* (LA 2133) and *L. hirsutum* (LA 1777) resistances are clearly different from that observed in *L. pennellii* (LA 716). This indicates that either *F. o.* f. sp. *lycopersici* can adapt to an extreme range of environments, or these resistances are

Table 2. Wilt development after inoculation of the *Lycopersicon* accessions with race 3 of *Fusarium oxysporum* f. sp. *lycopersici*[a]

Species	Accession	Healthy (no.)	Stunted[b] (no.)	Wilted (no.)	Total plants (no.)
Controls					
L. esculentum (susceptible)	Bonny Best	0	0	31	31
L. pennellii (resistant)	LA 716	41	1	0	42
Accessions					
L. esculentum	LA 2121	0	0	36	36
var. *cerasiforme*	LA 1310	7	0	27	34
L. pimpinellifolium	LA 2097	12	4	5	21
	LA 1582	8	2	3	13
	LA 1670	16	9	10	35
L. cheesmanii	LA 1449	7	5	30	42
L. chmielewskii	LA 1306	12	7	33	52
L. parviflorum	LA 2133	63	3	0	66
L. hirsutum	LA 1777	45	2	0	47
	LA 2409	18	0	5	23
L. hirsutum var. *glabratum*	LA 1625	12	0	6	18

[a] Results of two sets of inoculations were pooled.
[b] Plants were categorized as stunted if they showed no external wilt symptoms and were much smaller than uninoculated controls.

Table 3. Frequency distribution of visual rating scores of race 3 resistances in interspecific BC_1 and F_2 progenies between *Lycopersicon esculentum* 'Bonny Best' and *L. hirsutum* (LA 1777) and *L. parviflorum* (LA 2133)

	Frequency distribution visual rating score[a]					
	Resistant		Susceptible[b]			
Genotype	1	2	3	4	5	Total
Controls						
Bonny Best (BB)-susceptible	0	0	3	1	99	103
L. pennellii (LA 716)-resistant	68	0	0	0	0	68
F_1 (BB × LA 716)-resistant	117	5	0	0	0	122
BB × (BB × LA 716)	32	7	5	4	11	59
F_2 (BB × LA 716)	155	17	8	0	14	194
L. hirsutum (LA 1777)						
LA 1777	29	28	17	3	0	77
F_1 (BB × LA 1777)	1	1	3	2	0	7
BB × (BB × LA 1777)[c]	0	0	1	11	58	70
F_2 (BB × LA 1777)	2	4	5	3	4	18
L. parviflorum (LA 2133)[d]						
LA 2133	45	9	2	0	0	56
BB × (BB × LA 2133)	0	0	0	0	12	12
F_2 (BB × LA 2133)	27	16	10	4	62	119

[a] Plants were dissected and scored according to a visual rating system based on the level of vascular browning observed (5); 1 = resistant to 5 = susceptible.
[b] Based on results of Bonny Best, LA 716, and Bonny Best × LA 716 controls.
[c] Because results from the three BB × (BB × LA 1777)-BC_1 progenies were similar, they were pooled for this analysis.
[d] Bonny Best × LA 2133 F_1 was not analyzed because of poor germination of seeds from this cross.

Table 4. Segregation analysis of race 3 resistances in interspecific BC_1 and F_2 progenies between *Lycopersicon esculentum* 'Bonny Best' and *L. hirsutum* (LA 1777) and *L. parviflorum* (LA 2133)

Genotype	Segregation analysis[a] (χ^2)							
	One Dom	One Rec	Two Dom Indep	Two Rec Indep	Two Dom Inter	Two Rec Inter	One Dom One Rec Indep	One Dom One Rec Inter
Expected ratio (resistant:susceptible)								
BC_1	1:1	0:1	3:1	0:1	1:3	0:1	1:1	0:1
F_2	3:1	1:3	15:1	7:9	9:7	1:15	13:3	3:13
Controls								
Bonny Best (BB)-susceptible								
L. pennellii (LA 716)-resistant								
F_1 (BB × LA 716)-resistant								
BB × (BB × LA 716)	6.1*[b]	...	2.5 NS	...	53.2**	...	6.1*	...
F_2 (BB × LA 716)	19.3**	419**	8.6**	159**	82.8**	2,250**	7.0**	622**
L. hirsutum (LA 1777)								
LA 1777								
F_1 (BB × LA 1777)								
BB × (BB × LA 1777)	70.0**	...	210**	...	23.3**	...	70.0**	...
F_2 (BB × LA 1777)								
L. parviflorum (LA 2133)								
LA 2133								
BB × (BB × LA 2133)								
F_2 (BB × LA 2133)	95.9**	7.9**	674**	2.80 NS	19.6**	181**	159**	23.6**

[a] χ^2 analyses were only conducted on progenies with a population greater than 50. Dom, dominant; Rec, recessive; Indep, independent—the two genes can confer resistance independently of one another; Inter, interaction—both genes are necessary for resistance.
[b] *, **Significant at the 0.05 and 0.01 levels, respectively. NS = not significant.

not the result of selection pressure on the host. A survey of pathogenicity of *F. oxysporum* in these regions and an analysis of the modes of action of the resistances could provide vital clues to better our understanding of the nature of plant disease resistance.

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