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RFLP mapping of *I1*, a new locus in tomato conferring resistance against *Fusarium oxysporum* f. sp. *lycopersici* race 1

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Summary. The inheritance and linkage relationships of a gene for resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 1 were analyzed. An interspecific hybrid between a resistant *Lycopersicon pennellii* and a susceptible *L. esculentum* was backcrossed to *L. esculentum*. The genotype of each backcross-1 (BC_1) plant with respect to its *Fusarium* response was determined by means of backcross-2 progeny tests. Resistance was controlled by a single dominant gene, *I1*, which was not allelic to *I*, the traditional gene for resistance against the same fungal pathogen that was derived from *L. pimpinellifolium*. Linkage analysis of 154 molecular markers that segregated in the BC_1 population placed *I1* between the RFLP markers *TG20* and *TG128* on chromosome 7. The flanking markers were used to verify the assignment of the *I1* genotype in the segregating population. The results are discussed with reference to the possibility of cloning Fusarium resistance genes in tomato.

Key words: *Lycopersicon esculentum* – *L. pennellii* – Fusarium wilt – RFLP – Disease resistance

Introduction

The response of tomato plants to the soil-borne fungus *Fusarium oxysporum* f. sp. *lycopersici* has been the subject of intensive research over the last 50 years and is a model system for coadaptive pathogen-host evolution (Walker 1971; Beckman 1987; Elias et al. 1990). Since the fungus is responsible for a severe disease in the warmer regions of the world, considerable effort has been directed towards the breeding of resistant tomato varieties (Walker 1971; Sarfatti et al. 1990).

Bohn and Tucker (1939) were the first to identify the dominant gene *I* in *L. pimpinellifolium* that confers resistance against *Fusarium oxysporum* f. sp. *lycopersici* race 1. This gene was assigned to chromosome 11 (Paddock 1950). Resistance to race 2 of the pathogen was found to be controlled by the dominant gene *I2*, which also originated from *L. pimpinellifolium* (Stall and Walter 1965, Cirulli and Alexander 1966), and was mapped to chromosome 11 relative to morphological markers (Laterrot 1976). The gene *I2* was later found to be tightly linked (no recombinants) to the DNA restriction fragment length polymorphism (RFLP) marker, *TG105B*, on chromosome 11 (Sarfatti et al. 1989). Race 3 of the fungus, which overcomes the resistance conferred by genes *I* and *I2*, was recently isolated in Australia, California, and Florida (Grattidge and O'Brien 1982; Davis et al. 1988; Volin and Jones 1982). Consequently, a new dominant resistance gene, *I3*, was identified and introgressed into *L. esculentum* from *L. pennellii* (McGrath et al. 1987). The new gene was mapped to chromosome 7 and located 2.5 cM from the isozyme marker *Got-2* (Bournival et al. 1989).

The task of mapping new genes to the tomato map was profoundly facilitated by the development of a "saturated" RFLP map (Tanksley et al. 1989). Using this technology it is now possible to follow, in a single population, the segregation of hundreds of DNA markers and genes of interest. Since the RFLP markers cover the entire genome, linkage relationships of target genes can be readily identified. In tomato, the following disease resistance genes have been mapped using RFLPs: *Tm2* (tobacco mosaic virus; Young et al. 1988), *I2* (Sarfatti et al.1989), *Mi* (root knot nematode; Klein-Lankhorst et al. 1990), *Sm* (*Stemphylium*; Behare et al. 1990). In

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this study we utilize the RFLP system to place a new locus for resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 1 on the linkage map.

Materials and methods

Plant material

The control cultivars used here to differentiate between *Fusarium oxysporum* f. sp. *lycopersici* races 1 and 2 were Marmande, which is susceptible to races 1 and 2 of the pathogen, and Rehovot 13, which is resistant to race 1 only.

For the allelism test the *L. esculentum* cultivar, M82-1-8, which harbors the race 1 resistance originating from *L. pimpinellifolium* (*I*), was crossed with the resistant *L. pennellii* (LA716), and the F_1 hybrid was selfed. The parents, the F_1 hybrid, and an F_2 of 253 seedlings were inoculated.

For the mapping study the *L. esculentum* cultivar Vendor, which is susceptible to race 1 of the pathogen, was crossed as the female parent with the resistant *L. pennellii* (LA716). The interspecific hybrid was backcrossed as the male parent to Vendor, 136 backcross-1 (BC_1) progenies were grown, and DNA was extracted from leaf tissue. Due to flower abnormalities, only 91 BC_1 plants were crossed as male parents with Vendor to produce backcross-2 (BC_2) populations.

RFLP analysis

DNA was extracted from the cultivar Vendor, *L. pennellii* (LA716), their interspecific hybrid and the BC_1 population. DNA isolation, restriction digests, electrophoresis on agarose gels, Southern blots, hybridizations, and autoradiography were as described by Bernatzky and Tanksley (1986a), except that filters were probed with random hexamer-labelled plasmid (Feinberg and Vogelstein 1983).

The genomic DNA was digested using one of the following restriction enzymes: EcoRI, EcoRV, DraI, HaeIII, XbaI. One hundred and forty-five RFLP markers (random tomato genomic and cDNA clones), covering the entire genome, were radiolabelled and hybridized individually to the appropriate filters based on the polymorphism. In the "Results" section we discuss only the markers relevant to the mapping of the Fusarium resistance gene; the complete list will be published elsewhere (M. Abu-Abied, in preparation). Isozyme analysis of *Got-2* and eight additional isozymes was as described by Zamir and Tal (1987).

Fusarium inoculation

The inoculum for race 1 was derived from a single spore culture of *Fusarium oxysporum* f. sp. *lycopersici* race 1 that was isolated from diseased plants of the susceptible cultivar Marmande. One day after emergence, seedlings were inoculated with a spore suspension of the pathogen using the root-dip technique (Alon et al. 1974). A minimum of 20 seedlings of each BC_2 progeny test population was inoculated with race 1. Disease symptoms were recorded for a period of 20 days and classified into two categories: healthy – no external or internal symptoms (e.g., xylem discoloration), and diseased – dry, wilted, or stunted with vascular browning. The proportion of diseased seedlings was calculated (disease rate) for each of the analyzed populations.

Linkage analysis

The MapMaker program (Lander et al. 1987) was used for mapping analysis by the Kosambi function. Statistical analysis was performed using the DataDesk computer program for the Macintosh (Velleman and Pratt 1989).

Table 1. Response of tomato accessions to *Fusarium oxysporum* f. sp. *lycopersici* race 1 and the proposed genotypes with respect to the loci *I* and *I1*

Accession	Diseased/Total (Number of seedlings)	Fusarium resistance genotypes
Vendor	32/32	*ii i1i1*
Marmande	75/77	*ii i1i1*
Rehovot 13	3/41	*II i1i1*
L. pennellii LA716	0/5	*ii I1I1*
F_1 (Vendor × LA716)	0/20	*ii I1i1*
M82-1-8	0/19	*II i1i1*
F_1 (M82-1-8 × LA716)	0/16	*Ii I1i1*
F_2 (M82-1-8 × LA716)	49/253	[a]

[a] Both loci are segregating

Table 2. Monogenic segregation ratios of chromosome 7 and 11 molecular markers and *I1* in the interspecific BC_1 population. Chi-square values for the joint segregation of the markers with *I1* are shown. e/e – homozygous for the *L. esculentum* allele; e/p – heterozygous

Locus	Mapping enzyme	e/e	e/p	χ^2 for 1:1 ratio	Joint χ^2 with *I1*
Chrom. 7					
TG199	EcoRI	57	75	2.4	30.8***
TG20	XbaI	57	67	0.8	42.3***
I1	–	51	40	1.3	–
TG128	DraI	53	74	3.5	55.3***
GOT-2	–	54	58	0.1	47.4***
TG183	HaeIII	60	73	1.3	51.0***
TG143	EcoRV	57	70	1.3	38.1***
TG166	DraI	52	61	0.7	21.3***
TG61	EcoRI	57	65	0.5	22.0***
TG13	DraI	59	71	1.1	14.0***
Chrom. 11					
TG194	DraI	34	49	2.7	7.6**
TG108	EcoRV	45	89	14.4***	5.9*
TG10	EcoRV	46	83	10.6**	2.6
TG36	EcoRI	38	80	14.9***	0.8
TG30	HaeIII	25	65	17.8***	2.0
TG105b	EcoRI	45	89	14.4***	0.2
TG26	HaeIII	53	81	5.9*	0.1
TG104	EcoRV	26	40	3.0	0.2

* Significant at the 5% level
** Significant at the 1% level
*** Significant at the 0.1% level

Results

Monogenic inheritance of Fusarium race 1 resistance

The cultivated parent was susceptible to *Fusarium* race 1, while the wild species and the F_1 were resistant, indicating complete dominance for the resistance gene (Table 1). As shown in Fig. 1, two groups of disease rates were obtained in the BC_1. The first group of 40 plants had a

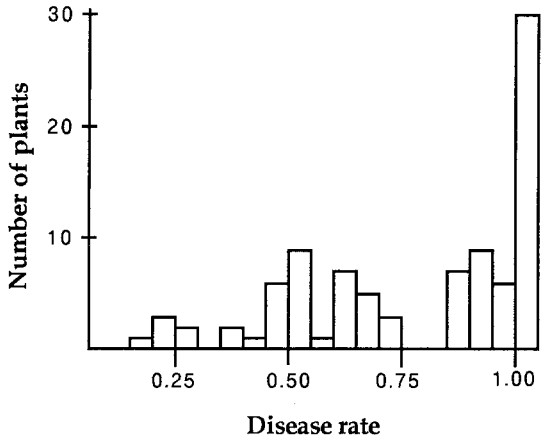

Fig. 1. Frequency distribution of Fusarium race 1 disease rate in the interspecific BC_1. Disease rate (the proportion of diseased seedlings) was determined by BC_2 progeny tests

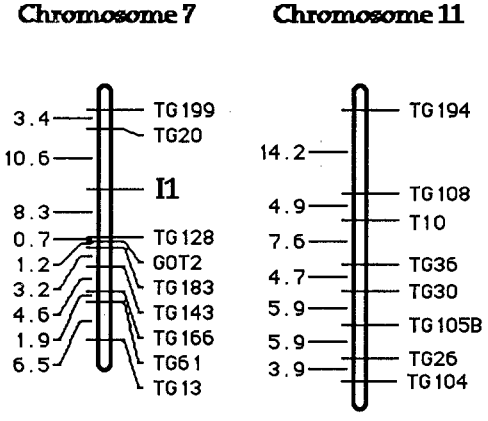

Fig. 2. Molecular maps of chromosomes 7 and 11

mean disease rate of 0.51 ± 0.15 (min. 0.18, max. 0.73) and was marked as heterozygous for the resistance. The second group of 51 plants, with a mean disease rate of 0.96 ± 0.05 (min. 0.85, max. 1.0), was marked as homozygous susceptible. The disease rate "cut-off point" between the two genotypes was confirmed by RFLP analysis. The ratio of homozygote to heterozygote genotypes in the BC_1 (Table 2) suggests that the *Fusarium* race 1 resistance that originated from *L. pennellii* is controlled by a single dominant gene.

The next step in the analysis was to determine if the gene *I* (conferring resistance to *Fusarium* race 1) that was introgressed into the cultivated tomato from *L. pimpinellifolium* is allelic to the race 1 resistance gene from *L. pennellii*. *L. esculentum* M82-1-8 was crossed with the homozygous resistant *L. pennellii*; the parents, the F_1, and F_2 of 253 seedlings were inoculated (Table 1). The proportion of susceptible plants was significantly higher than expected on the basis of the 1:15 ratio for two independent, dominant resistant genes ($P < 0.001$). Aberrant segregation ratios are common in this particular interspecific F_2 generation (Zamir and Tadmor 1986). The results indicate that the *I* gene derived from *L. pimpinellifolium* is not allelic to the resistance gene carried by *L. pennellii*, and we therefore propose the symbol *I1* for this gene. In Table 1, genotypes are assigned to the analyzed accessions with respect to both loci *I* and *I1*.

Linkage analysis

The segregation of 154 molecular markers, covering the entire genome (980 cM), was monitored in the interspecific BC_1. Association between *I1* and each marker was assessed using two-way contingency tables; significant deviations from the expected values were detected for markers mapping to chromosomes 7 and 11 (Table 2). The monogenic segregation ratios for markers from these chromosomes and the enzymes used for the RFLP mapping are recorded in Table 2. No significant deviations from the expected 1:1 ratio were calculated for the ten markers of chromosome 7, while for chromosome 11 six of the eight markers deviated significantly, favoring the heterozygous combination.

Highly significant associations were detected between *I1* and all chromosome 7 markers; for chromosome 11, *TG194* and *TG108* showed associations that were weaker than those of chromosome 7, yet were significant at the 1% level (Table 2). The mean disease rate of plants containing the *L. pennellii* allele at *TG194* was lower than that of the *L. esculentum* homozygotes (0.68 and 0.86, respectively; significantly different at the 1% level). The map positions of the chromosome 7 and 11 markers are shown in Fig. 2; *I1* mapped to chromosome 7 between *TG20* and *TG128*. The direction of the deviations from the expected Mendelian ratios for *I1*, as assayed in BC_2 progeny tests, was opposite to that of the linked markers *TG20* and *TG128* (assayed in BC_1), where an excess of heterozygotes was detected. This is because of the higher incidence of sterility in plants heterozygous for this segment of chromosome 7.

RFLP verification of the I1 scoring

The availability of RFLP markers on both sides of the resistance locus supported our *I1* genotype determination of the basis of disease rate for the BC_1 plants (Fig. 1). Analysis of 79 plants in which a complete data set was obtained for *TG20*, *I1*, and *TG128* is presented in Table 3. The percentage of recombination between *TG20* and *TG128* was 24.1, while the additive percentage of recombination including *I1* was 26.6. One individual that scored as heterozygous for *TG20* and *TG128* was found to be Fusarium susceptible (50 of 52 BC_2 progeny test seedlings were susceptible) and was therefore classified as a double recombinant. The possibility of seed contamination of this sample was reduced through isozyme anal-

Table 3. Joint segregation of *I1* and the flanking markers TG20 and TG128 for 79 BC_1 plants for which complete data were obtained

1st locus	2nd locus	A/A	A/B	B/A	B/B	Recombination fraction
I1	*TG20*	31	8	4	36	15.2
I1	*TG128*	36	8	1	34	11.4
TG20	*TG128*	29	11	8	31	24.1

A/A – a homozygous condition in the first and second locus; A/B – a homozygous condition in the first and a heterozygous condition in the second locus, etc.

ysis of the BC_2 seedlings for *Got-2* (which is linked to *I1*), *Pgm-2* (chromosome 4), and *Pgi-1* (chromosome 12). This double recombinant plant can explain the discrepancy between the two-point and the three-point test, indicating that the scoring based on disease rate was correct.

Discussion

High-resolution RFLP mapping of disease resistance genes is of interest for two main reasons. (1) Molecular markers flanking such genes can be used to increase the efficiency of simultaneous introgression of several targeted chromosome segments; (2) RFLP markers tightly linked to genes of interest can be used as starting points for physical mapping and chromosome walking aimed at cloning of the gene. Both of these applications require precise mapping of the factors responsible for the resistance.

Resistance, the ability of the host to suppress or retard the activity of the pathogen (Collinge and Slusarenko 1987), is a quantitative trait often determined by the activity of a single gene. Genetic mapping of a resistance gene requires transformation of the quantitative disease response into a simple Mendelian trait. The degree and severity of the Fusarium symptoms are affected by the host genotype, inoculum concentration, stage of seedling development, and soil temperature (Alon et al. 1974; Sarfatti et al. 1989). For these reasons, variations in the disease response may be observed for individuals with identical resistance genotypes. In order to assign Mendelian genotypes in experiments where large numbers of segregating progeny test populations are being evaluated, it is necessary to define the "cut-off points" within the disease rate distributions. Misclassification of a few individuals in the population can profoundly affect the position of the resistance gene relative to neighboring markers, particularly when saturated RFLP maps are used.

In a recent study which mapped *Fusarium* race 3 resistance to chromosome 7 (Bournival et al. 1989), five disease scales were scored in progeny test populations and a weighted mean disease rate was calculated. Because of the overlapping disease rate distributions obtained for the heterozygote and susceptible homozygote genotypes, it was difficult to classify some of the individuals. The present study demonstrates how flanking RFLP markers on both sides of the target gene can provide a way to confirm genotypic assignment. The three-point additive percentage of recombination (*TG20-I1-TG128*) was nearly identical with the percentage of recombination between *TG20* and *TG128*. The discrepancy (2.6% recombination) was explained by the presence of one double recombinant. Any misclassification would have resulted in a larger percentage of recombination in the three-marker analysis.

The additive distance between *TG20* and *TG13* in the BC_1 population was 37 cM, while a distance of 67 cM was previously observed between these markers in an F_2 generation involving the same interspecific cross (Zamir and Tanksley 1988). For chromosome 11, the distance between *TG108* and *TG104* was 33 cM in the BC_1 population and 56 cM in the F_2. These differences may be explained by higher rates of crossing-over for maternal gametes, which participate in the production of the F_2, as compared to the male gametes, which produce the BC_1. This phenomenon was previously demonstrated for two isozyme linkage groups in the same tomato cross (Gadish and Zamir 1987), and in a different tomato cross (Paterson et al. 1990).

Paddock (1950) was the first to assign a Fusarium-immunity locus against race 1 of the pathogen to chromosome 11: this locus, however, is still not placed on the map. In our study we detected an association between *TG194* and the disease response, suggesting that *I* may be linked to this marker. The locus *I1* that maps to chromosome 7 appears no to be allelic to *I3*, which maps to the same chromosome (2.5 cM from *Got-2*; Fig. 2) and appears to confer resistance also to race 2 (Bournival et al. 1990).

In tomato, a number of Fusarium resistance loci have been located, some of them linked to RFLP markers (Sarfatti et al. 1990). This linkage may allow the cloning of resistance genes via different methods of chromosome walks. In such experiments, there is always some uncertainty about how to determine when the destination is reached. In the tomato genome, approximately 50% of the cDNA clones are present in two to five copies (Bernatzky and Tanksley 1986b), indicative of a considerable number of duplications in its evolutions. The mapping of a number of resistance loci, which evolved through duplications, may make it possible to identify a clone containing the Fusarium resistance gene through its mapping to the different independent Fusarium resistance loci.

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