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An RFLP marker in tomato linked to the *Fusarium oxysporum* resistance gene *I2*

M. Sarfatti [1], J. Katan [2], R. Fluhr [3], and D. Zamir [1]*

[1] The Hebrew University of Jerusalem, The Faculty of Agriculture, Department of Field and Vegetable Crops and the Otto Warburg Center for Biotechnology in Agriculture, P.O. Box 12, Rehovot 76100, Israel
[2] The Hebrew University of Jerusalem, The Faculty of Agriculture, Department of Plant Pathology and Microbiology, P.O. Box 12, Rehovot 76100, Israel
[3] The Weizmann Institute of Science, Department of Plant Genetics, P.O. Box 26, Rehovot 76100, Israel

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Summary. The locus, *I2*, which in tomato confers resistance against *Fusarium oxysporum* f. sp. *lycopersici* race 2, was introgressed into *Lycopersicon esculentum* from the wild species *L. pimpinellifolium* (P.I. 126915). We searched for restriction fragment length polymorphisms (RFLPs) between nearly isogenic lines (NILs) in clones that map to the region introgressed from the wild species. Since *I2* maps to chromosome 11, we used DNA clones from this chromosome as hybridization probes to Southern blots containing bound DNA of the NILs digested with 23 restriction enzymes. Of the 14 chromosome 11 clones, 9 exhibited polymorphism. These clones were further hybridized to "verification" filters that contained DNA from resistant and susceptible *L. esculentum* varieties digested with the enzymes that gave the polymorphism. One clone, TG105, was found to be associated with *I2;* 19 susceptible lines showed a different RFLP with this probe than 16 resistant lines, including the original *L. pimpinellifolium* accession used as a source for the resistance gene. These results together with our mapping analysis indicate that TG105 is closely linked to the resistance gene.

Key words: Tomato – *Fusarium oxysporum* f. sp. *lycopersici* race 2 – RFLP – Disease resistance

Introduction

In tomato (*Lycopersicon esculentum*), most genes for disease resistance are dominant and were introgressed from wild germplasm (Stevens and Rick 1987). Modern cultivars must combine multiple resistances, thus breeding involves extensive disease screening. To increase the efficiency of such programs, tomato geneticists have identified useful markers that are linked to some of the resistance genes.

Robinson et al. (1970) introduced a breeding stock that was anthocyaninless (*ah*) and resistant to tobacco mosaic virus (*Tm2*). Using this line, breeders can increase considerably the proportion of F_2 plants homozygous to *Tm2* by selecting seedlings with green stems. Rick and Fobes (1974) encountered linkage between *Meloidogyne incognita* resistance (*Mi*) and Acid phosphatase-1 (*Aps-1*), an electrophoretically detectable codominant isozyme. This linkage enables breeders to select for resistance at early seedling stages by isozyme analysis instead of by exposure to the parasite. In this case, the variant *Aps-1* and *Mi* alleles were introgressed as a genetic block from the wild green fruited species *L. peruvianum* (Medina and Stevens 1980).

DNA restriction fragment length polymorphism (RFLP) markers (Beckmann and Soller 1986; Tanksley et al. 1989) are potentially very powerful tools in linking disease resistance genes, for the following reason: numerous independent genetic clones can be obtained and exploited to define the genetically distinct elements from the wild tomato species that have been used as a source for resistance genes. Young et al. (1988) used isogenic lines to identify DNA markers tightly linked to the *Tm2a* gene in tomato. The strategy used for isolating such markers was by testing genomic clones as hybridization probes against Southern blots containing DNA from pairs of nearly isogenic lines (NILs) for *Tm2a* restricted with different enzymes. Since the gene was introgressed from *L. peruvianum* together with a residual segment of the foreign chromosome, clones that were located on it exhibited RFLPs between the lines. Clones which are tightly linked to *Tm2a* can now be used for early screen-

* To whom correspondence should be addressed

ing of tobacco mosaic virus resistant lines (Young and Tanksley 1989).

The dominant gene *I* confers "immunity" against *Fusarium oxysporum* f. sp. *lycopersici* race 1. This resistance locus was first identified in *L. pimpinellifolium* (accession 160, P.I. 79532; Bohn and Tucker 1939). When race 2 of the pathogen first appeared (Alexander and Tucker 1945), a new dominant resistance gene (*I2*) was identified in a different *L. pimpinellifolium* accession (P.I. 126915; Stall and Walter 1965; Cirulli and Alexander 1966). *I2* was subsequently mapped to chromosome 11 on the classical tomato linkage map (Laterrot 1976). Recently, race 3 of the fungus which overcomes the immunity offered by genes *I* and *I2* was isolated in Australia and Florida (Grattidge and O'Brien 1982; Volin and Jones 1982). Consequently, a new dominant resistance gene *I3* was identified and introgressed into *L. esculentum* from *L. pennellii* (P.I. 414773; McGrath et al. 1987). In this paper we report the identification of an RFLP marker which is tightly linked to *I2*.

Materials and methods

Plant material. The origin of tomato varieties and breeding lines that were tested in the experiment are listed in Table 1. The NILs, Moneymaker and Motelle, were used for the initial search for polymorphic sites associated with the *F. oxysporum* race 2 resistant gene (*I2*). Plants from the original *L. pimpinellifolium* accession (P.I. 126915) used for the introgression of *I2* were also assayed for RFLP. For direct RFLP mapping, an experimental hybrid (HY9221) heterozygous for *I2* was selfed, and 31 F_2 plants were grown; F_3 seed were used for progeny tests of resistance or susceptibility to *Fusarium* wilt race 2 (an average of 30 seedlings peer F_2 plant).

RFLP analysis. DNA was extracted from each of the tomato lines and the F_2 population resulting from selfing of HY9221. Restriction digests, gel electrophoresis, Southern blots, hybridizations and autoradiography were as described by Bernatzky and Tanksley (1986).

The NILs genomic DNA was digested with the following 23 restriction enzymes: PvuII, PstI, HindIII, BamHI, AvaI, BglI, BglII, EcoRI, XbaI, XhoI, CfoI, BclI, BstEII, HpaII, MspI, EcoRV, RsaI, HincII, AluI, HinfI, TaqI, DraI and HaeIII. Fourteen DNA markers which map to chromosome 11 (Zamir and Tanksley 1988) were radiolabeled and hybridized individually to screen the NILs filters. Probes showing RFLPs between Moneymaker and Motelle were hybridized to "verification" filters containing DNA from the resistant and susceptible lines listed in Table 1. DNA from each segregant in the F_2 population was restricted with TaqI and hybridized with TG105, a clone which was produced from a sheared DNA library of tomato (Zamir and Tanksley 1988).

Fusarium inoculation. The inoculum was derived from a culture of *Fusarium oxysporum* f. sp. *lycopersici* race 2 that was isolated from diseased plants of the cultivar Rehovot 13, which is resistant to race 1 but not to race 2 of the pathogen. One day after emergence, tomato seedlings were inoculated by a culture suspension of the pathogen using the root-dip technique (Alon et al. 1973). The inoculated plants were surveyed for 20 days after inoculation and were visually classified into two categories: healthy – no external or internal symptoms (xylem discoloration), and diseased – completely dry, wilting or stunted with vascular browning.

Table 1. *Fusarium oxysporum* race 2 susceptible and resistant tomato lines: their sources and their TG105 genotype. (E = *L. esculentum* allele, 0.8-kb TaqI fragment; P = *L. pimpinellifolium* allele, 1.1-kb TaqI fragment; P/E = heterozygote)

Susceptible cultivars	Seed source	Diseased/ Total	TG105
Moneymaker	1	99/103	E
Vendor	2	15/ 20	E
Vendor F	2	16/ 20	E
Vendor VF	2	18/ 20	E
Vendor Tm-2a	2	7/ 13	E
Vendor VFT	2	7/ 12	E
Gardner	2	9/ 12	E
Gardner VF	2	10/ 12	E
Marmande	1	123/139	E
Marmande VF	1	7/ 10	E
Monalbo	1	8/ 12	E
Monita Moneymaker	1	12/ 14	E
Mocimor	1	7/ 12	E
Rehovot 13	3	87/ 92	E
Faculta 111	3	11/ 12	E
Alta	6	9/ 12	E
VFNT Cherry	4	11/ 12	E
LA1113	4	12/ 12	E
LA1643	4	10/ 12	E
Total		478/551	
Resistant cultivars			
Motelle	1	5/120	P
Ideucenzi	1	3/ 60	P
Hofit	6	2/ 15	P
Peto-95-43	6	2/ 19	P
Peto-94-C	6	1/ 19	P
Peto-81	6	0/ 12	P
Peto-434	6	0/ 12	P
Contendor	5	1/ 12	P
Holit	6	2/ 12	P
Jackpot (hybrid)	6	1/ 12	P/E
Duchess (hybrid)	6	1/ 08	P/E
7117 Cherry	6	0/ 14	P
Improved MH1	6	1/ 14	P
Bonanza (hybrid)	6	2/ 14	P/E
Celebrity (hybrid)	6	3/ 15	P/E
Horizon	6	0/ 13	P
Total		24/371	

Seed sources: 1–H. Laterrot, INRA, Avignon, France; 2–M. Mutchler, Cornell University, Ithaca, New York; 3–N. Kedar, Hebrew University, Rehovot, Israel; 4–C.M. Rick, University of California, Davis, USA; 5–D.J. McGrath, Bowen Horticultural Research Station, Australia; 6–commercially available seed

Results and discussion

The relative orientation of the classical (Stevens and Rick 1987) and RFLP (Zamir and Tanksley 1988) maps of chromosome 11 (Fig. 1) was not known at the initiation of the experiment and, therefore, 14 DNA markers that map to the same chromosome were screened for polymorphism between the NILs, Moneymaker and Motelle. Nine of 14 markers showed RFLP between the lines with at least one enzyme (Table 2), indicating that the lines are not truly isogenic. There was no indication of a particular enzyme being more informative than the others in showing RFLPs.

To determine which of the nine markers is associated with *I2*, the DNA probes were hybridized to "verification" filters that contained DNA from different resistant and susceptible lines digested with the restriction enzymes that generated the polymorphism. Only the polymorphism for TG105 was found to be consistently associated with the response of the plants to the pathogen. Nineteen susceptible lines were homozygous to a TaqI low molecular weight fragment of 0.8 kb, while 16 resistant lines and the original *L. pimpinellifolium* accession had a higher molecular weight TaqI fragment (1.1 kb; Table 1). Four of the cultivars that were F_1 hybrids showed heterozygosity for TG105.

To estimate the linkage between TG105 and *I2*, we carried out mapping analysis on a population of 31 F_2 plants whose genotype at TG105 was determined using the polymorphism provided by TaqI digestion (Fig. 2; Table 3); the flanking markers, TG46 and TG36, were monomorphic in this cross. The response of the plants to *Fusarium oxysporum* race 2 was determined based on F_3 progeny tests. Due to the incomplete penetrance of the resistance trait (Alon et al. 1973; Jones and Crill 1974), a phenomenon that was also detected in our inoculation experiments (6.5% of the 371 resistant seedlings developed symptoms, while 13% of the 551 susceptible seedlings escaped infection and were scored as healthy; Table 1), we classified the F_2 plants as either being resistant (homozygous *I2I2* and heterozygous *I2i2*) or susceptible (*i2i2*). No recombinants were detected between TG105 and *I2*. Recombination frequencies between TG105 and *I2* were estimated by the maximum likelihood equation (Allard 1956) to be between 0.0 and 4.8 centimorgans (cM). This estimate is supported by the lack of detectable recombination between the loci in the 16 independently bred resistant cultivars in Table 1. The mapping results appear to be consistent with the orientation of the chromosome 11 maps as drawn in Fig. 1.

The gene *I2* is linked to a number of easily distinguishable recessive morphological markers (Fig. 1; Laterrot 1976). The genetic stock LA1113 is homozygous for the markers anthocyaninless (*a*), blind (*bl*) and fasciated (*f*) and also susceptible to *Fusarium* race 2 (*i2i2;* Table 1);

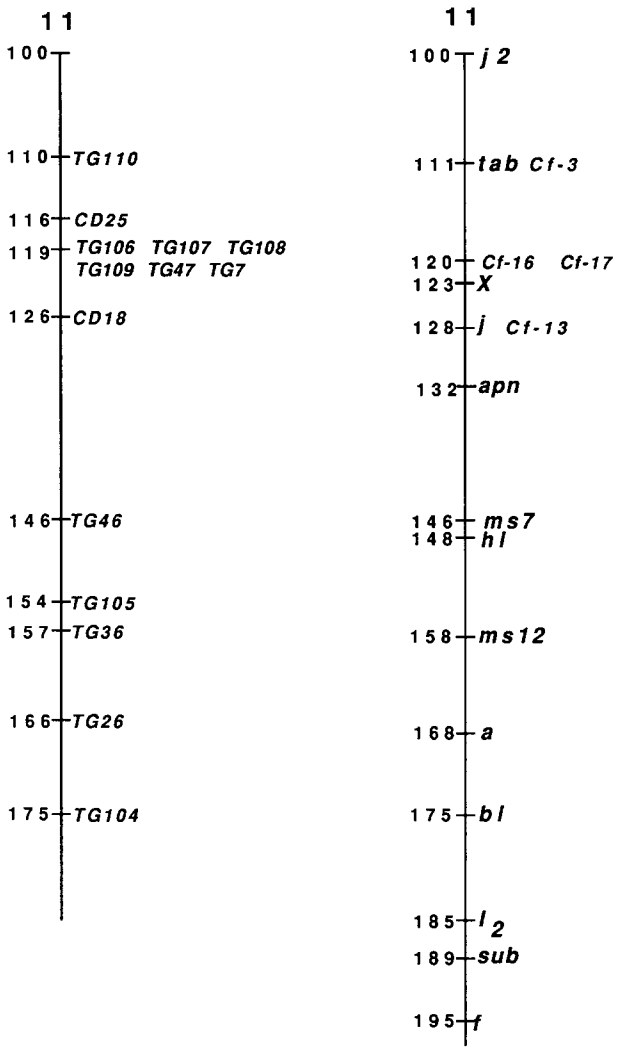

Fig. 1. Maps of RFLP (Zamir and Tanksley 1988) and morphological markers (Stevens and Rick 1987) of chromosome 11 of tomato. Distances on the map are in centiMorgans

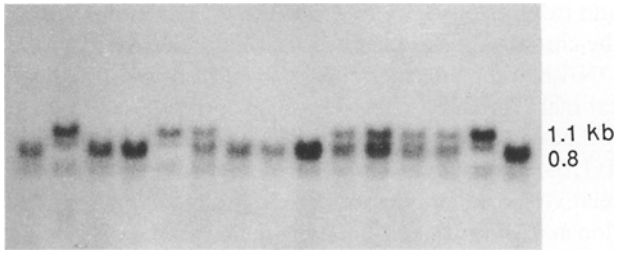

Fig. 2. Segregation of the RFLP marker TG105 in a population segregating for the *Fusarium* race 2 resistance. The Southern blots contained genomic DNA from 15 plants digested with Taql, and hybridized to the radiolabeled DNA clone TG105

LA576 is homozygous for the marker subtilis (*sub*) and susceptible to *Fusarium* race 2. To produce a high resolution map of TG105 relative to *I2*, we are currently analyzing F_2 populations resulting from hybrids between the morphological stocks and Motelle (*I2I2*), which carries

Table 2. Chromosome 11 DNA markers and the RFLPs detected between the nearly isogenic lines Moneymaker and Motelle

DNA marker	No. of restriction enzymes[a]	Polymorphic restriction sites
TG110	23	9
CD25	10	0
TG47	18	1
TG7	23	6
TG109	19	0
TG108	17	2
TG107	12	3
TG106	20	2
CD18	18	0
TG46	18	0
TG105	23	2
TG36	21	1
TG26	21	4
TG104	20	0

[a] Only restriction enzymes digests showing clearly distinguishable fragments were counted

Table 3. Genotypic frequency of TG105 and *I2* in the F_2 segregating generation after inoculation with *Fusarium* race 2 (E = *L. esculentum* allele, 0.8-kb TaqI fragment; P = *L. pimpinellifolium* allele, 1.1-kb TaqI fragment; P/E = heterozygote)

	TG105		
	P/P	P/E	P/E
Resistant *I2*	8	7	0
Susceptible *i2i2*	0	0	16

Map distance = 0.0 +/−4.8 cM

the dominant alleles of the morphological markers. Segregants that are homozygous for the recessive markers and exhibit resistance to *Fusarium* are recombinants for the chromosome segment separating these genes. As the DNA marker is located between one of the morphological markers and the resistance gene, the selected plants will be enriched for recombination events between *I2* and TG105. Using this approach, an RFLP analysis of a relatively small population should produce a high resolution map of the region spanning the target gene *I2* and its associated RFLP markers.

The advantage in application of RFLP markers which are tightly linked to resistance genes in breeding programs is manifested in the ability to efficiently screen genotypes without resorting to test inoculation with the pathogen. In addition, tightly linked RFLP clones may also function as starting points for various molecular approaches, such as chromosomal walks aimed at direct cloning of disease resistance genes (Michelmore et al. 1987, Young et al. 1988; Ellis et al. 1988).

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