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Mapping of *Ve* in tomato: a gene conferring resistance to the broad-spectrum pathogen, *Verticillium dahliae* race 1

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Abstract The soil-borne fungi *Verticillium* spp. cause vascular wilt disease in a wide range of crop plants. In tomato, resistance to *Verticillium dahliae* race 1 is conferred by a single dominant gene, *Ve*. Previous efforts to map *Ve* in tomato have yielded confusing results, locating it on different chromosomes, which subsequently raised the possibility that *Verticillium* resistance may be controlled by a number of loci. We used three different mapping populations to obtain an unambiguous map location of *Ve*: a recombinant inbred (RI) line population; an F_2 population segregating for *Verticillium* resistance; and a population of 50 introgression lines (IL). In all of the mapping populations *Ve* was positioned on the short arm of chromosome 9 tightly linked to the RFLP marker *GP39*. This linkage was confirmed by screening for *GP39* in different breeding lines with known resistance or susceptibility to *Verticillium*. A perfect match was found between *GP39* and the *Verticillium* response of the lines, indicating the potential of *GP39* in the rapid detection of *Verticillium* resistance and as a starting point for map-based cloning of *Ve*. This approach is particularly relevant for *Verticillium dahliae* race 1, since in the present work we also show that the isolate that infects tomato is responsible for wilt disease in other important crop plants.

Key words Linkage analysis · Mapping populations · Introgression Lines · RFLP · Host range

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Introduction

The soil-borne fungi *Verticillium dahliae* and *V. albo-atrum* cause vascular wilt disease in a wide range of crop plants and weeds throughout the world (Watterson 1986). Among the most important cultivated plants which are attacked by *Verticillium* spp. are: cotton (*Gossypium* spp.), tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), alfalfa (*Medicago sativa* L.), cucurbits, strawberry (*Fragaria grandiflora* Ehrh.), mint (*Mentha piperita* L.), sunflower (*Helianthus annuus* L.) and eggplant (*Solanum melongena* L.) (Cirulli 1981). Vascular wilt diseases such as *Verticillium* and *Fusarium* are among the most difficult plant diseases to control. To date, the most effective means of controlling them is the use of resistant varieties. Many crop species, such as cotton, alfalfa and mint contain genes for tolerance, but not complete resistance, to *Verticillium* spp. (reviewed by Hastie and Heale 1981). One of the exceptions is tomato in which resistance to *Verticillium dahliae* race 1 is conferred by a single dominant gene, *Ve*. *Ve* was identified in 1932 in an *L. esculentum* accession called Peru Wild, and the first resistant varieties were released in the 1950s (Schaible et al. 1951). Today, *Ve* is carried by most modern commercial tomato varieties.

Efforts at mapping *Ve* in tomato have yielded confusing results: the first suggested location was on chromosome 4 where *Ve* was found to be linked to the locus *e*, for entire leaf (Rick et al. 1959). Kerr and Busch

(1977) mapped *Ve* on chromosome 12, 39 units away from *alb*, a chlorophyll-deficiency locus. More recently, Juvik et al. (1991), using a recombinant inbred line population, suggested that *Ve* was linked to the restriction fragment length polymorphism (RFLP) marker *TG20* on chromosome 7, and Zamir et al. (1993) suggested that *Ve* resides on chromosome 9. These results gave rise to the possibility that, contrary to what was thought previously, *Verticillium* resistance may be controlled by a number of loci located on different chromosomal segments in the tomato genome. Resistance responses which are controlled by several loci, major and minor, are known for several plant diseases. One example is the tomato resistance to bacterial wilt (BW), a disease caused by *Ralstonia solanacearum*. In two studies, in which different mapping populations, resistance sources and infection methods were used, the same region on chromosome 6 was shown to have a major role in the resistance response (Danesh et al. 1994; Thoquet et al. 1996). However, minor effects by other genomic regions on chromosomes 4, 7 and 10 were also correlated with the resistance in those studies. Another example of multigenic resistance is the tolerance in tomato to the tomato yellow-leaf curl geminivirus (TYLCV). Using several mapping populations, Zamir et al. (1994) mapped a major incompletely dominant gene for TYLCV tolerance (*Ty*1) to chromosome 6 and two additional minor loci on chromosomes 3 and 7.

The objectives of the study presented here were to resolve the contradicting mapping results by screening a number of independent segregating populations of diverse origin and to determine the pathogeniety range of the tomato isolate of *Verticillium dahliae* race 1.

Material and methods

Plant material

Plants that were used to study the host range of *Verticillium dahliae* race 1 isolate were: (1) eggplant (*Solanum melongena*) cv 'Kelpic'; (2) melon (*Cucumis melo* L.) cv 'Ofir'; (3) cotton (*Gossypium hirsutum*) cvs 'Gedera 144' and 'Hazerah 23'; (4) pepper (*Capsicum annuum* L.), cv '1005'; (5) *L. esculentum* cv 'Microtom', a dwarf tomato and (6) control lines for inoculation – *L. esculentum* cv 'Mogeor' (*Ve*/*V*e), *L. esculentum* cv 'P-40' (*ve*/*ve*) and *L. esculentum* cv 'M-82' (*Ve*/*V*e).

The following mapping populations were inoculated with the same isolate described above:

1) a recombinant inbred (RI) population that resulted from an interspecific cross between UC204 C (*Ve*/*Ve*) X *L. cheesmanii* (*ve*/*ve*). The RIs are described by Paran et al. (1995);

2) F_3 progeny of an F_2 population resulting from a cross between *L. esculentum* cv 'Mottelle' (*Ve*/*Ve*) and *L. esculentum* cv 'Moneymaker' (*ve*/*ve*)). The F_2 population consisted of 46 plants;

3) introgression lines (IL) from the end of the short arm of chromosome 9: IL 9-1, IL 9-1-1, IL 9-1-2, IL 9-1-3 and IL 9-2 (Eshed and Zamir, 1995);

4) sixty two commercial cultivars and breeding lines known to be susceptible or resistant to *Verticillium*, including: 11 varieties susceptible to *Verticillium*, 30 resistant; and 21 commercial hybrids resistant to *Ve*.

Verticillium inoculations

The inoculum was obtained from a culture of *Verticillium dahliae* race 1 that was isolated from diseased susceptible tomato plants (test cultivar 'P-40'). Between 20 and 40 seedlings of the different species, cultivars, breeding lines, segregating populations and ILs were inoculated; the seedlings had completely open cotyledons and up to one true leaf. The roots of the seedlings were trimmed, dipped in a culture suspension of the fungus and planted in sterile soil. Three to four weeks after inoculation, the plants were scored for disease symptoms on a scale from 0 to 3: 0 = no internal or external symptoms; 1 = no external symptoms, some xylem browning; 2 = some external symptoms (wilting, drying, growth retardation), xylem browning; and 3 = severe wilting, extensive vascular browning usually accompanied by considerable growth retardation. Plants which scored distinctive levels of 2 or 3 were considered to have severe disease symptoms and therefore susceptible to *Verticillium*.

DNA extraction, Southern hybridization and linkage analysis

Procedures for DNA isolation, Southern blotting and hybridization were as described by Paran et al. (1995).

The RFLP map of the introgression lines was constructed by Paran et al. (1995). Additional markers that were not previously mapped in this population were positioned relatively to the existing markers by using the MAPMAKER program (Lander et al. 1987).

Results and discussion

RI mapping

Ve was first positioned in a recombinant inbred (RI) population (Paran et al. 1995). The disease response was unequivocally characterized as either homozygous resistant (*Ve*/*Ve*) or homozygous susceptible (*ve*/*ve*) in 58 lines. Our inability to characterize the entire population was due to the high residual heterozygosity in the population (Paran et al. 1995), and the possible presence of *Ve* modifier genes. *Ve* showed the tightest linkage, 10.1 cM, with the RFLP marker *GP39*, thereby positioning it on the short arm of chromosome 9 (Tanksley et al. 1992). These results are in agreement with those reported by Zamir et al. (1993), in which *TG105B*, a marker located on the short arm of chromosome 9, showed strong linkage with the resistance.

F_2 population

A new independent segregating population was used to verify the position of the *Ve* locus and its linkage with the RFLP marker *GP39*. An F_2 population between the resistant variety 'Mottelle' (*Ve*/*Ve*) and its near-isogenic susceptible variety 'Moneymaker' (*ve*/*ve*) was developed. The F_2 population was progeny-tested by

Table 1 Linkage relationships between the *Ve* locus and the RFLP marker *GP39* as calculated using an F_2 population resulting from a *L. esculentum* cv 'Mottelle' (*Ve*/*Ve*) × *L. esculentum* cv 'Moneymaker' (*ve*/*ve*) cross

Tested loci		Genotype of F_2 plants[a]					Total	Distance (cM)
First locus	Second locus	A/A	H/A	H/H	H/B	B/B		
GP39	*Ve*[b]	11	1	18	3	13	46	4.3

[a] A, Homozygous condition to the *L. esculentum* cv 'Mottelle' allele; B, homozygous condition to the *L. esculentum* cv 'Moneymaker' allele; H, heterozygous condition; A/A, homozygous condition to the *L. esculentum* cv 'Mottelle' alleles in both loci; H/B, heterozygous condition in the first locus and homozygous condition to the *L. esculentum* cv 'Mottelle' allele in the second locus, etc

[b] *Verticillium* inoculation results are based on F_3 progeny test of the F_2 population

inoculating F_3 plants with the pathogen and then probing them with chromosome 9 markers. Eight restriction enzymes were used in an attempt to map the markers that are known to be located on the short arm of chromosome 9 (Tanksely et al. 1992). However, excluding *GP39* which was polymorphic using the restriction enzyme *DraI*, none of the markers were polymorphic between 'Mottelle' and 'Moneymaker' (*GP39* reveals identical polymorphism as the RFLP marker *TG654*). *GP39* showed close linkage to *Ve* with only four recombination events between the two loci (Table 1), verifying the map location that was shown by the RI population, where a different resistance source was used.

ILs

In order to obtain further support for the position of *Ve* we carried out an analysis of resistant and susceptible ILs (Eshed and Zamir 1995). This population of 50 ILs originated from a cross between the green-fruited wild species *L. pennellii* and the cultivated tomato *L. esculentum* cv 'M82'. Each of the lines contains a single RFLP-defined chromosomal segment of *L. pennellii*. The lines are a set of near-isogenic lines to 'M82', and together completely cover the tomato genome.

ILs from the short arm of chromosome 9, IL 9-1, IL 9-1-1, IL 9-1-2, IL 9-1-3 and IL 9-2, were inoculated with the pathogen *Verticillium dahliae* race 1 (Fig. 1). ILs 9-1, 9-1-1 and 9-1-2, which contain introgressions of *L. pennellii* covering the telomeric end of the chromosome 9 short arm, showed a susceptible response to *Ve* ranging from 72.8% to 82.9% susceptible plants. That response was similar in magnitude to that of the susceptible control (Table 2). IL 9-1-3 and 9-2, which do not contain *L. pennellii* introgressions in that region, were resistant to *Ve*. The resistance response of IL 9-1-3 was similar to that of 'M-82', the resistant parent, while IL 9-2 showed a somewhat lower level of resistance. The strongest evidence for the location of *Ve* is the inoculation results of IL 9-1-3; this line identical to the susceptible line IL9-1 except that it does not include *GP39* (Fig. 1). IL 9-1-3 is resistant to *Ve*, indicating that *Ve* is tightly linked to the RFLP marker *GP39* and

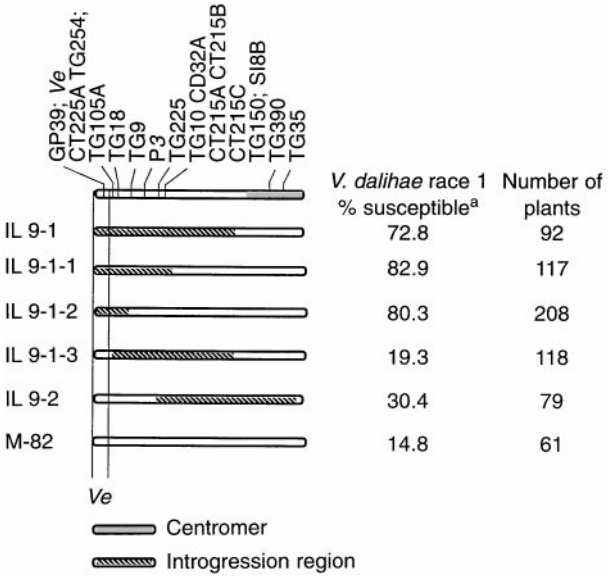

Fig. 1 Susceptible or resistant introgression lines (*IL*) to *Verticillium dahliae* race 1 on the short arm of chromosome 9

consequently positionning *Ve* on the short arm of chromosome 9 at its telomeric end.

Commercial cultivars

To further test the association of *GP39* with the *Verticillium* response, we probed commercial varieties with known resistance or susceptibility to *Ve* (see Materials and methods) with *GP39*. *GP39* showed a perfect match with the *Verticillium* response phenotype, indicating that a single *Ve* locus is present in the tomato genome and located on the short arm of chromosome 9. *GP39* has, therefore, a potential use in the easy and rapid detection of lines which are resistant to *Verticillium*.

Host range

Verticillium dahliae is known to have a wide host range (Cirulli 1981). We tested the host range of the tomato isolate of *V. dahliae* race 1, by inoculating a range of

Table 2 Results of inoculations of tomato lines and cultivars of other species with *Verticillium dahliae* race 1. Observations were made 3 weeks after inoculation (*S* susceptible, *R* resistant)

Plant	Disease visual rating (no. of plants)				Total no. of plants	Percentage susceptible[a]	*Ve* response
	0	1	2	3			
P-40 (*L. esculentum*)	7	12	18	65	102	81.4	S
Mogeor (*L. esculentum*)	65	20	19	5	109	22.0	R
Microtom (*L. esculentum*)	23	15	21	18	54	72.2	S
Pepper (1005)	46	8	10	6	70	22.9	R
Sp.Eggplant (Kelpic)	10	25	39	35	109	67.9	S
Melon (Ofir)	4	3	10	56	73	90.4	S
Cotton (Gedera 144)	1	6	17	54	78	91.0	S
Cotton (Hazerah 23)	0	19	47	43	109	82.6	S

[a] Number of plates with a disease rating of 2 and 3, total number of plants

crop species. Of the crops that were tested only pepper showed partial resistance to the pathogen, as compared with the resistant tomato variety, 'Mogeor' (Table 2). The pepper plants did not show any apparent typical disease symptoms such as browning of the xylem and wilting. However, growth of the pepper plants was suppressed as compared to the control uninfected pepper plants. Scoring of the pepper plant for the absence or presence of the disease was therefore based only on the size of the plants. All of the other species tested showed clear symptoms of infection by the tomato isolate of *V. dahliae*, although their level of susceptibility and the type of symptoms varied. In all cases, the diseased plants were compared to healthy uninfected controls that were grown simultaneously in the greenhouse. The cotton plants showed very clear, easy to detect browning of the base of the stem. Browning signs were not obvious for melon where the base of the plant sometimes appeared yellow. However, indications of wilt in the melon plants were very clear and included wilted leaves and stems. Eggplant and tomato showed identical symptoms – browning of the xylem, wilt and growth retardation.

Overall, the percentage of susceptible plants varied among the species from 68% in eggplant to 91% of the cotton plants. In all cases, when plants showed disease symptoms, the *V. dahliae* fungus could be re-isolated from these plants. These results suggest that the fungus *Verticillium dahliae* race 1 has a wide spectrum host range. It causes wilt disease not only in tomato, but also in other important crops such as cotton, melon and eggplant.

Conclusions

This study indicates that the *Ve* gene that confers full resistance to the fungus *Verticillium dahliae* race 1 in a wide range of cultivated tomato genotypes is located at a single locus near the telomeric end of the short arm of chromosome 9, tightly linked to the RFLP marker *GP39*. These results exclude the possibility that *Verticillium* resistance is control by a multi-loci system, such as shown for BW resistance (Danesh et al. 1994; Thoquet et al. 1996) and TYLCV tolerance (Zamir et al. 1994) in tomato. The differences in the map location of *Ve* between earlier reports (Rick et al. 1959; Kerr and Busch 1977) and the current one may be due to the lack of a phenotypic marker at the top of chromosome 9 (Rick 1977). In contrast, the location of *Ve* on chromosome 7 (Juvik et al. 1991) was probably due to the fact that the RFLP markers of the tightest linkage to *Ve*, *TG105* and *GP39*, were not tested in that study. In any case, we cannot eliminate the possibility that other genetic modifiers of *Verticillium* resistance response are located elsewhere in the tomato genome and that the locations of *Ve* suggested by other groups may be of such modifiers.

Recently, co-dominant and allelic-specific sequence-characterized amplified region (SCAR) markers were developed for *Ve* by Kawchuk et al. (1998). The RFLP marker *GP39* together with the SCAR markers could be used as a starting point for map-based cloning of the *Ve* gene (Tanksley et al. 1995).

Cloning of the *Ve* tomato gene may offer a biotechnological tool for inducing resistance in other susceptible crop species which do not have a mono-genic dominant resistance to *V. dahliae*.

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