MPMI Vol. 9, No. 9 1996, pp. 826-836. Publication no. M-1996-1028-04R. © 1996 The American Phytopathological Society

Quantitative Trait Loci Determining Resistance to Bacterial Wilt in Tomato Cultivar Hawaii7996

Philippe Thoquet, Jocelyne Olivier, Christoph Sperisen, Peter Rogowsky, Henri Laterrot, and Nigel Grimsley

Laboratoire de Biologie Moléculaire de Relations Plantes-Microorganismes, CNRS-INRA, B.P. 27 Auzeville, 31326 Castanet-Tolosan, France
Received 26 March 1996. Accepted 4 Sepember 1996.

Several loci governing resistance to bacterial wilt, a disease caused by *Ralstonia solanacearum*, have been mapped in tomato (*Lycopersicon esculentum*). An F_2 population derived from a cross between a highly resistant cultivar, Hawaii7996, and a very susceptible line of *L. pimpinellifolium*, WVa700, was used to develop a map of molecular markers. The parental lines were screened for polymorphism using 462 RFLP probes with 14 restriction enzymes. Genetic mapping revealed that the distribution of polymorphisms was very uneven. Nevertheless, the extensive screening permitted development of a map covering much of the genome. Nine independent resistance tests were done on cuttings from plants of this population in a controlled environment culture chamber. In addition to the most important QTL detected on chromosome 6, analyses of our data using either nonparametric or interval mapping tests suggest the presence of another QTL on chromosome 4 (2 QTL) and weaker putative QTL at other map positions. Together these loci, all derived from the resistant parent Hawaii7996, account for 30 to 56% of the phenotypic variation observed.

Additional keywords: Burkholderia solanacearum, genome, *Pseudomonas solanacearum,* tolerance.

Bacterial wilt caused by *Ralstonia solanacearum* (previously named *Pseudomonas solanacearum*, then reclassified as *Burkholderia solanacearum*, and recently as *R. solanacearum* (Yabuuchi et al. 1992, 1995)) is one of the most important bacterial plant diseases in the world, affecting hundreds of different species, mainly in tropical and subtropical climates (Kelman 1953), including many crops such as potato, tomato, eggplant, pepper, ground nut, and banana. The bacterial parasite was first isolated in culture over a hundred years ago (Smith 1896), and five races are now classified according to host range (Buddenhagen et al. 1962). The bacteria enter the roots at sites of secondary root emergence (Kelman and Sequeira 1965; Schmit 1978) or at root tips (Vasse et al. 1995) and progress to the xylem, then spreading systemically in the plant. Large numbers of bacteria secreting exopolysaccharides and proteins involved in pathogenesis then impair water transport and lead to disease symptoms in the host, finally ending in wilting and death (Buddenhagen and Kelman 1964).

Although a hypersensitive type of response to *R. solanacearum* has been observed in some species (Carney and Denny 1990; Arlat et al. 1994), there is no clear genetic evidence for gene-for-gene resistance. In tomato, the bacterial parasite is able to invade even the most resistant cultivars of the plant, where it resides apparently without producing symptoms; the plant response of limiting the invasion is thus more accurately described as tolerance (Grimault and Prior 1993). Nevertheless, we henceforth use the more general term "resistance" since many plant breeders associate "tolerance" with some adverse effect on the plant, which may not be the case for the variety Hawaii7996 in the field.

We chose tomato as a model to study bacterial wilt because a dense restriction fragment length polymorphism (RFLP) map from an interspecific cross with *L. pennellii* has been established (Tanksley et al. 1992), different resistant and susceptible lines are available, and a great deal of work has already been done on the physiology of the response to the disease.

Resistance to bacterial wilt was difficult to combine in certain breeding programs with the resistance to nematodes conferred by the gene *Mi* (Acosta et al. 1964), suggesting that one important locus may reside on chromosome 6. Recently this observation was confirmed in other lines of tomato (Prior et al. 1994) and by mapping with molecular markers (Aarons et al. 1993; Thoquet et al. 1993; Danesh et al. 1994). In addition, QTL on chromosomes 7 and 10 were identified as being involved with resistance in a cross between L285, resistant (*L. esculentum* var. *cerasiforme*) and CLN286, susceptible (Danesh et al. 1994).

Using molecular markers on an F_2 population derived from a cross between a highly resistant tomato *L. esculentum* var. Hawaii7996 (H7996) and a highly susceptible wild tomato line *L. pimpinellifolium* WVa700 (WVa700), we report here on the identification of genetic loci that may be involved in determining the resistance.

Corresponding author: N. Grimsley; E-mail: grimsley@toulouse.inra.fr

Present address of C. Sperisen: Swiss Federal Institute for Forest, Snow and Landscape Research, Zürcherstrasse 111 CH-8903 Birmensdorf, Switzerland.

Present addresses of Peter Rogowsky: RCAP, ENS-Lyon, 46 Allée d'Italie, 69364 Lyon cedex 07, France.

Present address of Henri Laterrot: INRA, Station d'Amélioration des Plantes Marachères, Domaine Saint-Maurice, BP 94, 84143 Montfavet, Avignon, France.

RESULTS

Analysis of polymorphism.

A total of 462 RFLP probes were tested on DNAs of the two tomato parental lines digested with 14 different restriction enzymes. These probes can be arbitrarily divided into two kinds: (i) tomato probes that were previously mapped in an interspecific cross between *L. esculentum* and *L. pennellii* (Tanksley et al. 1992), and (ii) a selection of potato probes and several cDNA of genes of known functions that have not been previously mapped in tomato. Potato probes were mapped previously in interspecific crosses of *Solanum*, allowing their likely map positions to be estimated before mapping them in our material, since the potato and tomato genomes are largely collinear, differing by five inversions (Bonierbale et al. 1988; Gebhardt et al. 1989; Gebhardt et al. 1991).

The level of polymorphism found between H7996 and WVa700 is similar whatever kind of probe is used (cDNA or genomic DNA of the different species), about 6% of the probes/enzymes combinations are polymorphic, giving 19.4% of polymorphic probes.

The parental lines were screened for polymorphisms using 300 arbitrary RAPD primers. Considering that each primer amplifies an average of five DNA fragments, about 1,500 bands were compared between the lines, and the level of useful polymorphism was quite low (3 to 4%) as with the RFLP markers.

Synthetic oligonucleotides, complementary to several different possible kinds of minisatellite, were hybridized to DNAs of the two lines digested with enzymes as described above. Amongst those tested, only the sequence $(GATA)_4$, giving complicated but polymorphic band profiles, was useful. The other minisatellite sequences, giving simpler band profiles, did not reveal polymorphisms.

About 500 loci were screened with 462 RFLP probes (some probes hybridizing to more than one locus), giving an approximate coverage of one marker every 3 cM, assuming a total map length of 1,276 cM (Tanksley et al. 1992).

Polymorphisms were not distributed randomly and chromosomes could be grouped into four classes according to the percentage of probes showing polymorphisms: (i) chromosome 6 (32%), (ii) chromosomes 5, 10 (≈20%), (iii) chromosomes 1, 2, 4, 7, 9, 11 (≈10%), and (iv) chromosomes 3, 8, 12 (≈3%). In addition, some chromosomal segments are more polymorphic than others. Thus, the region 0 to 60 cM of chromosome 6 is very polymorphic, and certain zones of other chromosomes are relatively polymorphic (region 0 to 40 cM of the chromosome 4). In the same way, most of the RAPD markers map in the chromosome 4 polymorphic zone. The map constructed with the markers reflects this nonhomogenous distribution (see Fig. 1).

Genetic map of Hawaii7996 × WVa700.

Eighty eight out of 462 RFLP probes revealed polymorphisms. Sixty of these were useful for mapping, the rest giving bands either too close together or too weak to score reliably in our gel systems. The useful probes are comprised of 16 tomato cDNAs, 20 tomato genomic clones, 6 potato cDNA

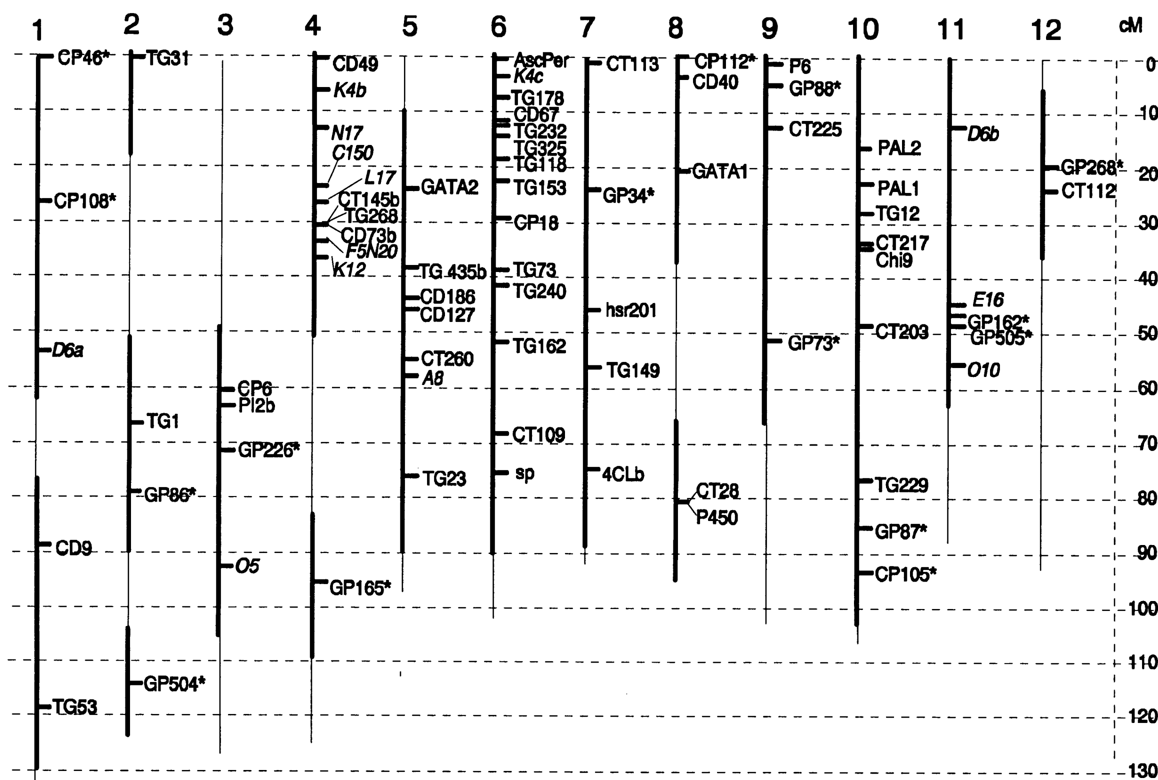

Fig. 1. The H7996 × WVa700 map is indicated by bold vertical lines. Map positions of probes are indicated by horizontal traits. Thin vertical lines represent tomato chromosomes described previously (Tanksley et al. 1992). Distances (in centiMorgans) are calculated using the software JOINMAP (P. Stam, 1993). Chromosomes are identified by previously mapped probes (Tanksley et al. 1992; Gebhardt et al. 1991). Potato markers are indicated by asterisks. Markers in italics are RAPD.

clones, 11 potato genomic DNAs, and 9 cDNAs of known sequence. Thirty five RAPD probes were also analyzed on the population, and 13 of these were useful for mapping. Several polymorphisms were noted using the $(GATA)_4$ probe. Two of these dominant polymorphisms were mapped on chromosomes 5 and 8.

The above markers, 75 in total, were used to make a genetic map (Fig. 1) using the software JOINMAP and the Kosambi mapping function. Linkage groups were assigned using previously mapped probes (Tanksley et al. 1992) as landmarks. Markers have been mapped also using the Mapmaker software and result is similar (data not shown).

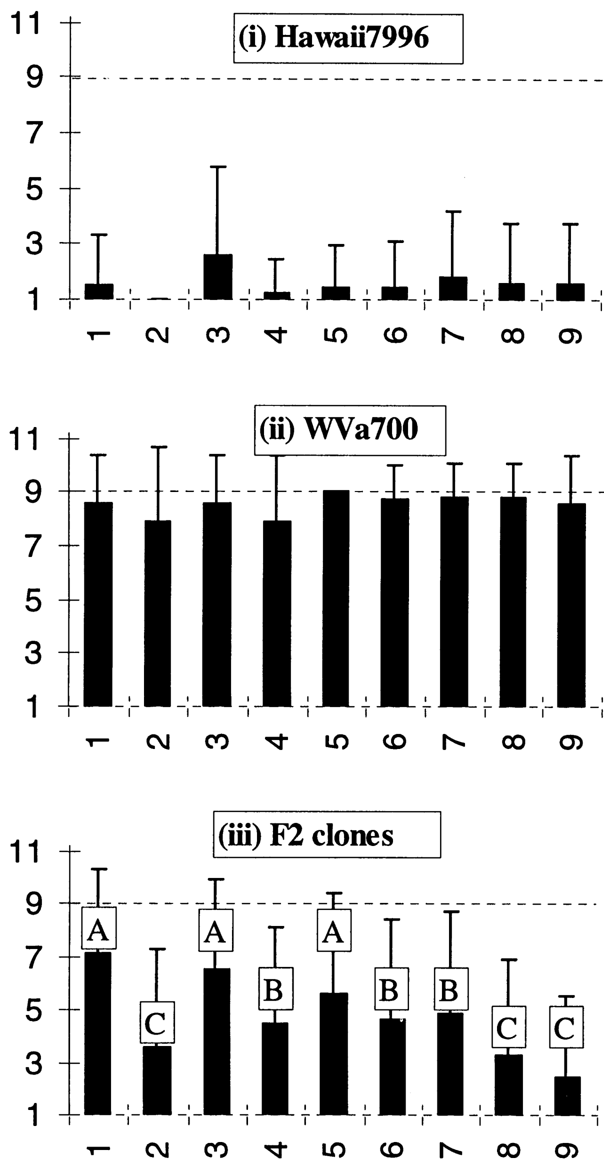

Fig. 2. Mean disease index scores in nine independent tests of the F_2 population compared to control parental lines. Tests (abscissa, chronological order, 1 = January 1993, 2 = July 1993, 3 = October 1993, 4 = November 1993, 5 = February 1994a, 6 = February 1994b, 7 = February 1994c, 8 = March 1994, 9 = May 1994) were classified into three groups A, B, and C as shown according to severity of the tests (ordinate scale 1 to 9, standard deviations indicated, Materials and Methods).

The map positions of some of the probes (CT145, CD73, TG435, TG325, PI2, CP112, and 4CL) do not agree with previous work (Tanksley et al. 1992). Our map position for TG325 agrees with Van Ooijen et al. (1993). CP112 hybridizes to many bands and already maps to several loci in potato, so its new position is not surprising. PI2 is also present in several copies and one locus was previously mapped in an interspecific cross on chromosome 11 (Taylor et al. 1993). CD73, CT145, CD49, and TG268, are part of the same linkage group in our study, whereas the former two probes were previously mapped elsewhere (Tanksley et al. 1992). In this case, CD49 and TG268 provide landmarks on chromosome 4 since the distance between them agrees with previous work (Tanksley et al. 1992). TG435 was mapped to chromosome 6 (Tanksley et al. 1992), but in our map it is linked to TG23 on chromosome 5. Probes often hybridize to more than one genomic DNA sequence, and it is therefore not surprising that some new loci are found.

Some probes were not linked to any other marker and were positioned with respect to the previously published tomato or potato map.

The total genetic distance between polymorphic probes mapped in the cross is 600 cM, a half of the map published recently (Tanksley et al. 1992). However, if both linked and

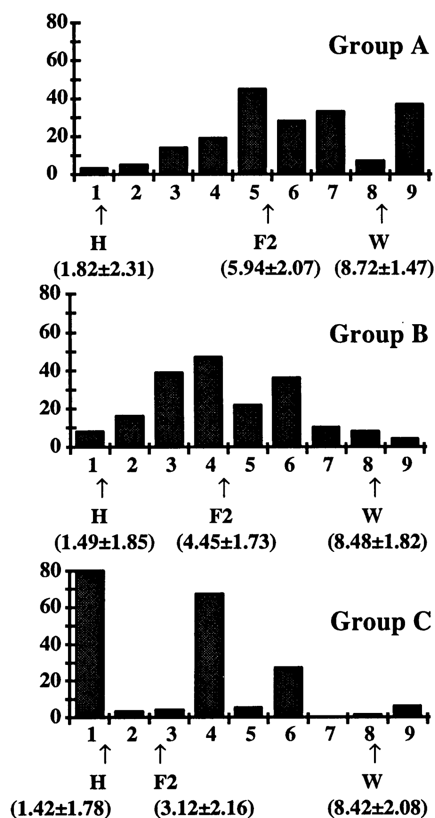

Fig. 3. Distribution of disease index frequency classes of the F_2 population within groups. Abscissa: classes of disease index (1: resistant plant, 9: susceptible plant). Ordinate: number of F_2 lines. Results were grouped into three lots, each representing three independent tests, according to the severity of disease symptoms: **A,** severe, **B,** intermediate, and **C,** mild. H: H7996, W: WVa700, F_2: F_2 population. Numbers in parentheses show the means and standard deviations of the bacterial wilt response in the F_2 population and in the parental controls.

unlinked markers analyzed in this study are considered, about 1,000 cM of the tomato genome could be analyzed for linkage to resistance to *R. solanacearum*. This value is estimated by summing the intervals between all of the linked markers and segments of 15 cM around the unlinked markers and represents about 75% of the tomato genome.

Distortion of segregation.

In the map, some markers show a distorted segregation. This is the case for the markers on chromosome 11 which show an excess of H7996 alleles ($\chi^2 = 17.2$ for GP162). There is also a deficit of H7996 genotypes on the upper arm of chromosome 2,($\chi^2 = 26.6$ for TG31). The marker GATA, on chromosome 5, shows a distorted segregation, although it showed a Mendelian segregation in a previous study (Ruskortekaas et al. 1994).

Genetic analysis of resistance following clonal propagation of F_2 plants.

Resistance tests were done on plants from rooted cuttings of the population, to permit the resistance of clones of one individual to be assayed over several different tests, and to allow the analysis of subsequent populations. The analysis was performed on a cross between the two lines showing the most susceptible and resistant phenotypes in our hands, WVa700 and H7996.

Nine tests were done in the growth chamber in Toulouse, each test with one cutting of each the 200 F_2 individuals and with 40 cuttings of each of the two parental lines. Although H7996 showed a good level of resistance, in certain tests, a proportion of WVa700 plants survived and variation was noted in the response of the F_2 population between tests (Figs. 2 and 3).

If all of the tests are compared using the levels of wilting found at the end of each test by analyses of variance, then three groups of "severity" can be distinguished (groups A, B, and C; Fig. 3, and Materials and Methods).

In group A, comprising three tests in which the plants were most severely attacked, the mean of the disease index of the F_2 population is 5.94 ± 2.07, and a rather bimodal frequency distribution of disease severity is observed (Fig. 3). The distribution in group B, where an intermediate level of severity is observed, is closer to normal, and the mean is 4.45 ± 1.73. The distribution in group C, comprising three tests with the lowest proportion of diseased plants, is more discrete, giving 4 main classes: 80 plants always resistant, 67 plants that die 1 out of 3 times, 27 plants that die 2 out of 3 times, and 7 plants that die in all tests of this group, giving a mean of 3.12 ± 2.16 (Fig. 3).

Since resistance to bacterial wilt did not segregate in a simple Mendelian way in the majority of the population that we studied, the character was treated as quantitative and analyzed using two different softwares: Map QTL (Van Ooijen and Maliepaard 1996), which permits either a nonparametric analysis of data or interval mapping, and Mapmaker/QTL (Lander et al. 1987) which permits interval mapping. Since the latter two kinds of analyses gave essentially similar results, only analyses performed using MapQTL (Van Ooijen and Maliepaard 1996) are presented.

The results were first analyzed with a nonparametric rank sum test (Kruskal and Wallis test). Analyses of the groups described above show several loci involved in bacterial wilt resistance (Table 1). A region chromosome 6 (between TG118 and CP18) is strongly associated with the phenotype in all groups ($P < 0.001$). This is the same for the upper arm of chromosome 4 (near TG268), but with a less significant association with data of group C ($P = 0.05$). On the same chromosome, marker GP165 presents association with results of group A ($P = 0.05$) and of group B ($P < 0.001$), but not with group C. Results of group A show also association with chromosome 11 (near GP162, $P = 0.005$). On chromosome 10, marker CP105 is weakly associated with resistance only with data from group B ($P = 0.05$).

As a comparison, interval mapping was also performed on the data. Using the group A data, the strongest effect is again observed on chromosome 6, over a large region around CP18 (LOD = 9). QTL are also observed on chromosome 4 on the upper arm around TG 268 and on the lower arm around GP165, group B data showing the strongest effect around TG268 (LOD = 4.5). Group A also shows a weak effect on chromosome 11 (LOD = 2.4). However, this result must be interpreted with caution since there is a strong distortion in favor of H7996 in this region, which might be a reason for this association.

Dominance.

The contribution of the different QTL to phenotypic variation was estimated using the SAS software (Materials and

Table 1. The Kruskal-Wallis test statistic for 32 markers spanning the tomato genome[a]

Chromosome	Marker	Group A	Group B	Group C
1	CP108	2.87	0.15	0.11
	CD9	3.68	2.28	0.25
2	TG31	0.08	1.22	0.09
	GP86	0.27	4.19	1.29
	GP504	4.75	1.65	2.89
3	GP226	1.41	0.72	2.33
4	CD49	3.94	8.88 a	3.22
	TG268	12.66 c	16.05 e	6.03 a
	TG464b	0.33	0.83	0.13
	GP165	9.00 a	18.31 e	2.41
5	TG435	0.23	2.26	4.87
	CD127	0.81	0.00	1.26
	TG23	1.85	0.21	3.07
6	TG178	7.87 a	15.37 e	10.94 c
	TG118	21.85 e	29.60 e	19.34 e
	CP18	27.08 e	26.35 e	14.50 d
	CT109	10.17 b	5.34	1.71
7	CT113	3.73	0.86	0.58
	GP34	0.10	0.26	2.05
	hsr201	1.90	0.30	2.44
	TG149	4.41	0.52	1.90
8	CD40	0.28	3.88	2.94
	CT28	0.08	0.10	0.16
9	GP73	0.40	0.39	0.75
	CT225	2.54	1.66	0.95
	P6	2.24	1.08	1.44
10	PAL2	0.12	0.14	0.53
	CT126	2.68	0.69	1.47
	TG229	0.96	2.01	5.43
	CP105	3.16	6.56a	5.80
11	GP162	11.27 c	5.84	2.37
12	GP268	2.91	0.00	0.61

[a] Letters after numers indicate linkage of the marker with the tolerance phenotype at the following confidence levels: a: $P = 0.05$, b: $P = 0.01$, c: $P = 0.005$, d: $P = 0.001$, e: $P < 0.001$.

Methods). The ratio of the variation due to dominance of one parental allele over another or to additive effects between the alleles was calculated (Table 2). Small values of this statistic indicate that most of the variation is additive (and therefore useful for selection), whereas larger values indicate that dominance plays a greater role. Negative values indicate that the Hawaii7996 (resistance) allele is recessive. At all of the identified QTL, only the H7996 alleles contribute to the resistance, WVa700 alleles show no positive effect on the phenotype. Neglecting between-locus interactions and ignoring overestimations that may arise by choosing those markers that show the effects, analyses of all the loci associated with bacterial wilt resistance explain 56% of the total variation for group A, 42% for group B, and 30% for group C.

DISCUSSION

Since *L. esculentum* var. Hawaii7996 was the variety presenting the highest level of resistance in growth chambers, and in the field it has a also good level of resistance (Scott et al. 1993), and since little DNA polymorphism is found within the species *L. esculentum*, we first looked for a suitable wild tomato species that could be used for genetic analysis. Under culture chamber conditions the resistance of the very polymorphic susceptible wild species *L. pennellii*, which was used as a parent in the construction of a detailed genetic map (Tanksley et al. 1992), was difficult to score. A line of wild tomato, *Lycopersicon pimpinellifolium* WVa700, was the most susceptible line tested in the same conditions, and subsequent genetic analyses were therefore done on populations derived from the cross H7996 × WVa700. Unfortunately, later in the molecular analysis, the lines used in our study were found to have little polymorphism in many regions of the genome. In a previous study on *Lycopersicon* species (Miller and Tanksley 1990), the number of polymorphic probes or enzyme-probe combinations, was, respectively, 78 and 33% between *L. esculentum* and *L. pimpinellifolium,* whereas variation within *L. esculentum* was 20 and 6%. We observed the latter levels of polymorphism, suggesting that WVa700 is closer to *L. esculentum* than it is to *L. pimpinellifolium.*

Linkage map.

Tomato is one of the genetically best characterized plant species, with more than 1,000 RFLP markers on the genome spanning 1,400 cM (Tanksley et al. 1992). A good saturation of markers has been achieved, since four telomeric sequences have been shown to lie within 10 cM of the most distal markers (Ganal et al. 1992). Since the tomato and potato genomes are very similar (Bonierbale et al. 1988; Gebhardt et al. 1991), an additional 400 RFLP markers are available. Out of 462 RFLP markers tested for polymorphism, 62 were useful in our cross.

To increase the density of markers on the genome, several minisatellites and many RAPD markers were screened. Probes that recognize a number of DNA sequences dispersed throughout the genome are likely to reveal more polymorphisms than probes detecting single loci. This is useful when little polymorphism is available and when probes covering the whole genome are sought. Several highly repeated sequences have so far been characterized in tomato, among them TGRI, a telomeric sequence (Ganal et al. 1988), the subtelomeric sequence TEL (Ganal et al. 1992), and the minisatellites GATA and GACA (Vosman et al. 1992). We used $(GATA)_4$ as a probe and found several polymorphisms between our lines. In another tomato cross, this marker behaved in a Mendelian way, segregating as a dominant marker (Ruskortekaas et al. 1994). In our material, the GATA1 and GATA2 markers were also dominant, but GATA2 presented a distorted segregation. A linked marker, TG435, 23 cM distant, segregates normally, suggesting that the proximal chromosomal region is not subject to a general distortion. The distortion of GATA2 segregation might arise from the deleterious effect of a more distal locus or from mutations arising from unequal recombination. The frequency of mutations at loci containing the TGR I or the TEL sequences can be as high as 10% per generation (Broun et al. 1992), a level sufficient to explain the distortion observed in our population.

RAPD markers (Williams et al. 1990) have been used for phylogenetic studies (for example, Vierling et al. 1992; Kresovich et al. 1992), for mapping single genetic loci (Klein-Lankhorst et al. 1991; Martin et al. 1991; Michelmore et al. 1991) and for construction of genetic maps (Reiter et al. 1992). As polymorphisms can be detected rapidly with these markers, we assessed the possibility of using them in our analysis.

The proportion of primers reproducibly revealing polymorphisms between H7996 and WVa700 was 16%, or approximately 3% of bands screened. These results agree with observations on other lines of tomato (Foolad et al. 1993). However, we discontinued use of these markers because (i) they did not reveal more polymorphisms than RFLP probes, (ii) they are less informative because their dominance, and (iii) none have previously known map position in our cross.

Very little distortion of segregation was observed in the F_2 population, reflecting the close homology between these lines at the DNA level, despite their classification into two species according to fruit size. In contrast, interspecific crosses such as *L. esculentum* × *L. pennellii* show much greater levels of distortion in more numerous regions on the genome (Bernatzky and Tanksley 1986; Kinzer et al. 1990). Some of

Table 2. Statistical results for markers of the tomato genome associated with bacterial wilt disease response in the F2 population

		Group A		Group B		Group C	
Chromosome	Marker	d/a[a]	Variation (%)[b]	d/a	Variation (%)	d/a	Variation (%)
4	TG268	−1.28	8.8	-0.95	10.9	-1.24	6.6
4	GP165	0.10	6.1	0.26	10.4	–	–
6	TG118	0.18	20.3	0.48	21.4	0.33	17.7
11	GP162	1.17	10.4	–	–	–	–

[a] Ratio of dominance to additive effects

[b] Percentage of total variation in bacterial wilt response explained by a quantitative trait locus at the marker.

these distortions can be explained by the presence of an incompatibility system in the species *L. peruvianum* (Tanksley and Loaiza-Figueroa 1985), but others, often involving similar genomic regions in different crosses, remain unexplained (Zamir and Tadmor 1986; Gadish and Zamir 1987; Kinzer et al. 1990).

We detected a reduction in the number of H7996 alleles on chromosome 11, a region also showing distortion in two other crosses between *L. esculentum* and *L. pimpinellifolium* (Behare et al. 1991; Segal et al. 1992). In the latter cases, loci determining resistance to *Stemphylium* or to *Fusarium oxysporum*, both being derived from *L. pimpinellifolium*, were being mapped, and it is therefore likely that a genetic factor controlling this distortion is specifically found in crosses with this species.

Out of all of the different kinds of markers tested, 77 were useful for mapping, allowing linkages over nearly 1,000 cM to be detected. Since the majority of markers that we mapped were already positioned in tomato or in potato (Tanksley et al. 1992, Gebhardt et al. 1989, 1991), new markers were easily assigned to linkage groups. Other studies using a similar number of polymorphic markers observed about the same genetic length of genome in an intraspecific cross (Danesh et al. 1994) or in an interspecific cross (Paterson et al. 1988).

Resistance to *R. solanacearum*.

Recently, several genes that behave as monogenic dominant genetic traits conferring specific resistance to pathogens have been characterized at a molecular level (Panaccione et al. 1992; Bent et al. 1994; Jones et al. 1994; Whitam et al. 1994; Mindrinos et al. 1995; Grant et al. 1995; Song et al. 1995), and most of these genes are probably involved in signal transduction mechanisms. In our material, resistance to bacterial wilt segregates in a complex way, the different tests revealing that at least four different loci may be involved under the conditions reported. The resistance to *R. solanacearum* is therefore most likely fundamentally different from that found in gene-for-gene systems, and may resemble some other polygenic resistances (for example, Leonards-Schippers et al. 1994; Bonierbale et al. 1994; Li et al. 1995; Sandbrink et al. 1995), where individual loci contribute to an increased level of resistance. In our material, all of the loci involved in the resistance come from the variety H7996, simplifying interpretation of the results.

The locus on chromosome 6 may be of general importance for resistance to *Ralstonia solanacearum*, since previous studies have found resistance to bacterial wilt is difficult to combine with resistance to nematodes encoded by the gene *Mi*, which maps to chromosome 6 (Acosta et al. 1964). An equivalent locus was detected in another tomato variety, L285, on genetic analysis in a cross with a susceptible line (CLN286), with a different strain and environmental conditions (Danesh et al. 1994). Although there is no indication in the literature that the resistance of L285 and of H7996 originate from a common progenitor, we cannot exclude this possibility. However, while the locus accounts for over 50% of the variability observed in the L285 × CLN286 cross, it accounts for only 20% of the variability in our material (Materials and Methods). Since the QTL detected in our study extends over a large region of the chromosome, we do not know whether one or more genes are involved, and further work must be done to resolve this question. It is interesting that a gene controlling this QTL lies close to the different loci controlling resistances to a plethora of other diseases in this chromosomal region (Klein-Lankhorst et al. 1991; Jones et al. 1993; Williamson et al. 1994; Vanderbeek et al. 1994; Kaloshian et al. 1995). The genetics of resistance to bacterial canker, another disease causing wilting symptoms, in *Lycopersicon peruvianum* segregates as several QTL, and again one of the QTL maps to chromosome 6 in this region close to TG178 (Sandbrink et al. 1995).

We find neither the effect on chromosome 7 noted in a previous study on bacterial wilt (Danesh et al. 1994)—perhaps because this effect was found only after introduction of bacteria to artificial wounds in the stem—nor the effect in the upper region of chromosome 10, although we do observe the presence of a minor QTL on the lower extremity of this chromosome under some conditions (Table 1). This result, however, must be interpreted with great caution since the confidence level ($P = 0.05$) can easily be found by chance considering that 12 linkage groups are being analyzed. In other cases, for example with GP165, the weak association found ($P = 0.05$) in group A is confirmed in the analysis of group B. Whereas the QTL on chromosomes 6, 4, and 11 are clearly confirmed by interval mapping, the weak effect found on chromosome 10 is not and thus remains in doubt.

We decided to use inoculation by adding a bacterial suspension to the roots, as this kind of inoculation mimics the natural situation. Entry of the pathogen into the plant is a very important part of the life-cycle of the interaction. Clearly, in this pathosystem exclusion of the pathogen by the resistant variety cannot explain the resistance, since resistant varieties are also invaded, but it is nevertheless possible given the complexity of the interaction that entry of the pathogen activates plant responses that differ between resistant and susceptible varieties in some way. However, it is also possible that different elements of the complex resistance can more easily be recognized by analyzing inoculations done with different techniques. Inoculation by root wounding or by stem puncturing gives very strong selection for resistant lines in field tests, and these techniques have been used in some programs (Winstead and Kelman 1952; Krausz and Thurston 1975; Mew and Ho 1977; Somodi et al. 1993). We chose to score the plants carefully on a 1 to 9 disease resistance scale, and we also attempted initially analyses using different statistics (lag period before onset of wilting, rapidity of wilting, and final level of wilting). However, the variation of these latter characteristics was very large, even in the control parental lines, and such data could not be used for analysis. Given the level of variation, a scale of 1 to 5 would have been adequate for the analysis.

Chromosome 4 plays a role in resistance in the majority of the tests done in Toulouse. Markers on the upper and lower arms show linkage to resistance. The upper arm is relatively polymorphic, since several randomly chosen RAPD markers and several new RFLP loci have been found here. Comparison of the polymorphisms found between H7996 and two susceptible lines, WVa700 and Floradel (*L. esculentum*) reveals that it is the resistant variety which contributes most to this high level of polymorphism, lending support to the notion that this region may have been introgressed from a progenitor resistant to bacterial wilt. Only one polymorphic probe has so far been

detected on the lower arm of chromosome 4, despite continued efforts to detect further polymorphisms, but linkage of resistance to this probe is detected in several tests. Since no centrally located marker linking the upper and lower arms of chromosome 4 has yet been mapped, it is possible that the effects found are part of one QTL. However, this hypothesis is unlikely because the effect on the upper arm is recessive, while on the lower arm it is dominant or codominant. In the absence of other markers on the lower arm of chromosome 4, GP165, a probe mapped so far only in potato, can only provisionally be positioned in this region using the colinearity with the potato genome as a guide for our analysis, and a slight doubt therefore remains about the position of the linked putative QTL. We are currently attempting to clarify this situation, but further efforts are limited by the lack of polymorphism in this region.

In view of the variation observed in the segregation of resistance in the clonally propagated F_2 population replicate tests, we thought it inadvisable to use selective genotyping as a means for increasing the population size screened using molecular markers. Selective genotyping depends on the accurate identification of resistant individuals, and is hindered by the presence of distorted segregations (Sandbrink et al. 1995). Our data may now permit an assessment of the use of selective genotyping for future work.

As noted in previous studies, many environmental factors influence resistance, and it will be interesting to attempt to define loci that play roles in different geographical locations where tomato production is of economic or social importance. Aggressivity differs markedly between bacterial strains, even among bacteria endemic to one geographic location (Prior et al. 1990). Temperature is probably one of the most important factors. Consequently, to conduct a genetic analysis of an F_2 population we chose to analyze the segregation of resistance using a very agressive strain in a cross between the most resistant and most susceptible genotypes available in culture chambers at a relatively high temperature to favor the development of disease symptoms, conditions allowing the best discrimination of resistant genotypes. Even in tropical regions where there is little difference between mean "winter" and "summer" temperatures the expression of resistance differs markedly between seasons, higher temperatures favoring development of the disease (Acosta et al. 1964; Sequeira and Rowe 1969, Krauz and Thurston 1975; Mew and Ho 1977; French and De Lindo 1982; Prior et al. 1990; Scott et al. 1993;). Since wilting is thought to result primarily from the blockage of water conducting vessels by masses of bacteria and by bacterial exopolysaccharides, the level of humidity could also influence the expression of disease symptoms. Young plants are more susceptible to the disease than older plants (Mew and Ho 1976; Nakaho and Takaya 1993; Perera et al. 1993), and the dominance of the resistance trait in some tomato varieties can alter with age and become recessive (Acosta et al. 1964). These observations may, in part, explain the reason for the heterogeneity of our resistance tests results when using plants grown from cuttings of the F_2 population. Although these individuals are of clonal origin, the cuttings were made over a period of 2 years in different seasons from aging mother plants. The large between-test variability in symptom severity led us to split the data into three groups (by the analysis of variance). Within these groups, only 30 to 56% of the total phenotypic variation was accounted for. The remaining variation could be due to environmental variation, or to loci whose effect is too weak to detect with the limited population size, or to loci present in unmarked regions of the genome. The most variation was detected in tests showing the most severe symptoms (group A) in keeping with the observation that the weaker putative QTL are not detected in less severe tests. This observation may be useful for designing resistance tests in the future. However, although the level of wilting in our F_2 population varied between tests, genetic mapping often gave concordant results concerning the location of the some of the loci affecting resistance.

In conclusion, further efforts are required to define which loci are important using different environmental conditions (in different worldwide locations) and to define more precisely the chromosomal regions involved in governing the resistance.

Although genetic analysis has permitted the detection of different loci important for resistance, the molecular basis for this character remains completely obscure. Different ways tackling this problem might now be envisaged. Genetically characterized individuals available from this study can now be used to create further lines that lack or possess known combinations of the different loci for molecular or cytological studies.

MATERIALS AND METHODS

Plant material.

Two genotypes, *Lycopersicon esculentum* cv. Hawaii7996 (H7996) resistant to *R. solanacearum* and *L. pimpinellifolium* cv. WVa700 (WVa700), susceptible, were maintained in the greenhouse after growing from seed and by propagation of cuttings. Two hundred F_2 seeds from a single F_1 plant from a cross H7996 × WVa700 were grown up and also maintained by cuttings in the greenhouse in Toulouse. Leaf tissue was harvested from parental, F_1, and F_2 plants, and used for DNA preparation.

Resistance tests.

In order to permit repetition of resistance tests on individual plants, all tests were performed on rooted cuttings from each F_2 (1 cutting/test) and from control parental lines (40 cuttings/test). Nine tests were done in a growth chamber in Toulouse. In all cases, control and tested plants were assigned individual numbers and distributed randomly to minimize environmental effects.

Cuttings were made from apical or side shoots having two leaves in individual pots with a mixture of compost/sand (2/1 v/v) under plastic cover in trays (40 pots/tray). After a week, the plastic was cut to reduce the humidity, and 5 days later it was removed. Rooting of different series' of cuttings, each series composed of about 200 individuals from the F_2 population and 40 individuals of each of the parental lines, was done in the greenhouse from about March to October, and in a culture chamber (16 h/8 h light/dark, 23°C/20°C day/night, 200 $\mu E\ cm^{-2}s^{-1}$) from November to March. When rooted, the cuttings were transferred to a growth chamber (light: 16 h, 30°C, 500 $\mu E\ m^{-2}\ s^{-1}$; dark: 8 h, 25°C) in a P3 containment laboratory, and allowed 1 week to acclimatize before inoculation.

Bacterial stocks were stored in glycerol at –80°C. The bacterial strain used throughout for inoculation was GMI8217 (A.

Trigalet, personal communication), resistant to streptomycin and rifampicin, a derivative of GT1 (Prior and Steva 1990). The strain was first grown on solid medium BGT (Boucher et al. 1985) at 30°C, and three single phenotypically mucous colonies were used to start liquid minimal medium cultures. After 36 h of culture at 30°C, the OD at 660 nm was measured, and the bacterial suspension diluted with distilled sterile water to a final OD of 0.1 (about 10^8 bacteria cm^{-3}).

In the culture chamber, each plant was inoculated with 50 ml of this bacterial suspension by pouring over the roots, and culture was then continued as above.

Disease symptoms were noted regularly during 30 days following inoculation using the following scale: 1—healthy, 2—a few leaflets show epinastic morphology, 3—one leaf wilted, 4—25% of the leaves wilted, 5—50% of the leaves wilted, 6—75% of the leaves wilted, 7—one leaf remaining healthy, 8—all leaves wilted but the plant apex remaining upright, and 9—completely wilted with the apex bending over.

Molecular analyses.

Standard protocols and abbreviations for solutions in general use in molecular biology are described elsewhere (Sambrook et al. 1989).

DNA extraction.

A modified phenol extraction procedure was used (Rogowsky et al. 1993). Two grams of freshly harvested leaves was immersed in liquid nitrogen and then ground to a fine powder in a precooled pestle and mortar. The powder was transferred to a 50-ml tube with 10-ml of extraction buffer and mixed; then 10 ml of phenol/chloroform 1:1 was added. After gentle agitation for 5 min at 4°C, the aqueous phase was separated by centrifugation (15 min, 1,800 × *g*) and transferred to a further tube where the phenol/chloroform extraction was repeated in a similar way. DNA was precipitated from the recovered upper phase by addition of 1.25 ml of 3 M sodium acetate and 10 ml of isopropanol at room temperature, spooled out, transferred to a new tube, washed once with 70% ethanol and twice with absolute ethanol. After drying at room temperature, the DNA was resuspended in TE containing ribonuclease (40 µg/ml). DNA concentration and quality were assessed by agarose gel electrophoresis in comparison with a standard series of lambda DNAs.

RFLP markers.

14 restriction enzymes (*Eco*RI, *Eco*RV, *Bam*HI, *Asp*700, *Hin*dIII, *Bgl*II, *Sca*I, *Dra*I, *Rsa*I, *Alu*I, *Sau*3A, *Taq*I, *Hae*III, and *Hin*fI or *Nde*II) were used independently on each of the parental genotype DNAs (H7996 and WVa700). Five- to ten-µg lots of DNA in 22 µl of buffer were digested (4 U enzyme/µg DNA, except for *Sau*3A [2U/µg DNA]) in the conditions recommended by the supplier. Digestions were done for at least 2 h at 37°C (65°C for *Taq*I), then a similar amount of enzyme was added once more, and digestion continued for another 2 h. When useful polymorphisms appeared, 5-µg lots of DNAs from each F_2 plant (200 individuals) were digested with the appropriate enzyme in a similar way.

Separation of fragments was done on 0.7% (for *Eco*RI, *Eco*RV, *Bam*HI, *Asp*700, *Hin*dIII, *Bgl*II, *Sca*I digestions) or 1% (for *Dra*I, *Rsa*I, *Alu*I, *Sau*3A, *Taq*I, *Hin*fI, and *Hae*III digestions) agarose gels for 16 h using an electric field of 1 V/cm.

Transfer to Hybond-N+ (Amersham) membranes was done according to the supplier using 0.4 M NaOH for 4 h. Membranes were then washed with 5× SSC, and fixed to the membrane using U.V. (312 nm for 3 min). After prehybridization at 42°C for at least 6 h in 20 ml of hybridization buffer (5×SSPE, 5× Denhart, 30% formamide, 0.5% SDS, 0.2mg/ml single-stranded salmon sperm DNA) with shaking, hybridization was carried out using 30 ng of ^{32}P-labeled probe (previously prepared, see below, denatured by boiling) in a new lot of 20-ml hybridization buffer for at least 6 h at 42°C. The membranes were then washed (50 ml of 2× SSC for 5 min at 20°C, then 50 ml of 2× SSC, 0.1% SDS for 15 min at 20°C, then twice at 42°C for 30 min in 50 ml 0.5× SSC, 0.1% SDS).

Autoradiography was done at −70°C in cassettes with 2 amplification screens (Appligene) with Fuji RX film for 2 to 4 days after wrapping the membranes in cellophane film. Following exposition, the membranes were stripped of probe by boiling in a solution of 1 mM EDTA, 0.1% SDS, and stored damp at 4°C wrapped in cellophane film. Membranes were reutilized for hybridization with different probes up to 20 times.

As a source of putative RFLP markers, (i) 339 cloned tomato sequences (TG clones: genomic DNA, CT and CD clones: cDNA; (Tanksley et al. 1992); (ii) 98 cloned potato sequences (GP clones: genomic DNA, CP clones: cDNA; Gebhardt et al. 1989; Gebhardt and Salamini 1993) were generously provide by S. T. Tanksley and by C. Gebhardt, respectively. Some cDNA clones of known genes from other species were also used for RFLP screening.

RFLP probes were maintained in *Escherichia coli* strain DH5α at −70°C. Three-milliliter cultures of individual bacterial strains were grown overnight in LB at 37°C, boiled for 10 min, centrifuged (10,000 rpm, 10 min) and the supernatant was recovered. Five microliters of this was used for PCR amplification (Saiki et al. 1988), using 50 ng of an appropriate flanking primer (New England Biolabs, Inc.). Insert size was verified by electrophoresis.

After phenol/chloroform purification and ethanol precipitation, a 30-ng lot of amplified insert was taken to prepare a radioactive probe using to a ReadyToGo kit (Pharmacia). Unincorporated nucleotides were removed by column chromatography.

Minisatellite and random amplified polymorphic DNA (RAPD) markers.

Synthetic oligonucleotides ($(CT)_8$, $(CAG)_5$, $(TGT)_5$, $(CCGT)_4$, $(TTGT)_4$, $(GTCA)_4$, $(GATA)_4$, $(AAACCCT)_4$, Eurogentec, Belgium) were used to screen for polymorphisms (Vosman et al. 1992).

300 RAPD primers (Operon, USA and University of Vancouver, Canada) were also screened (Williams et al. 1990) on the parental lines. Putative polymorphic markers were retested on the parents, and then analyzed on a population of 92 F2 progeny.

Genetic analyses.

The segregation of RFLP, microsatellite, and RAPD markers was analyzed using the softwares MAPMAKER (Lander et al. 1987) and JOINMAP (Stam 1993). Markers were assigned to linkage groups using a threshold of LOD 3.

Resistance test results were compared to each other by analysis of variance by using the PROC procedure of SAS. Tests were grouped according to the proportion of wilted plants. Within groups, using the scores of individual cuttings, a mean disease index was calculated for each F_2 clone.

Results obtained with the groups determined previously were analyzed in relation with the genotype at different loci with the Kruskal-Wallis rank-sum test (see e.g., Lehmann 1975) or by interval mapping, using the MapQTL software (Van Ooijen and Maliepaard 1996).

ACKNOWLEDGMENTS

RFLP probes were generously provided by the laboratories of Christiane Gebhardt, Steve Tanksley, Pierre de Wit, Michel Delseny and Bernard Lescure. We thank Brigitte Mangin for advice on statistical analysis, and Matthieu Arlat for critical reading of the manuscript. Johan van Ooijen and Piet Stam allowed us to use softwares developed by their laboratories before these became generally available. Jenny Grimsley helped with some of the plant work and Jean-Luc Pariente assured continued support in the maintenance of plants in the greenhouse. Sarah Stephens and Guusje Bonnema helped with some of the initial work of this analysis. N.G. thanks Maarten Koornneef his practical introduction to tomato genetics. Peter Rogowsky and Christoph Sperisen received one-year fellowships from the European Community Human Capital and Mobility program (contract no. ERB4001GT910964) and the National Fonds Suisse, respectively. We thank the Région Midi-Pyrénées for providing part of the financial support (grant no. 3172A).

LITERATURE CITED

Aarons, S. R., Danesh, D., and Young, N. D. 1993. DNA genetic marker mapping of genes for bacterial wilt resistance in tomato. Pages 170-175 in: Bacterial Wilt. G. L. Hartman and A. C. Hayward, eds. ACIAR Proc. No. 45. Watson Ferguson Co., Brisbane, Australia.

Acosta, J. C., Gilbert, J. C., and Quinon, V. L. 1964. Heritability of bacterial wilt resistance in tomato. Proc. Am. Soc. Hortic. Sci. 84:455-462.

Arlat, M., Van Gijsegem, F., Pernollet, J. C., Huet, J. C., and Boucher, C. A. 1994 PopA1, a protein which induces a hypersensitive-like response on specific petunia genotypes, is secreted via the Hrp pathway of *Pseudomonas solanacearum*. EMBO J. 13:543-553.

Behare, J., Laterrot, H., Sarfatti, M., and Zamir, D. 1991 Restriction fragment length polymorphism mapping of the *Stemphylium* resistance gene in tomato. Mol. Plant-Microbe Interact. 4:489-492.

Bent, A. F., Kunkel, B. N., Dahlbeck, D., Brown, K. L., Schmidt, R., Giraudat, J., Leung, J., and Staskawicz, B. J. 1994 *RPS*2 of *Arabidopsis thaliana*: A leucine-rich repeat class of plant disease resistance genes. Science 265:1856-1860.

Bernatzky, R., and Tanksley, S. D. 1986 Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887-898.

Bonierbale, M. W., Plaisted, R. L., and Tanksley, S. D. 1988. RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095-1103.

Bonierbale, M. W., Plaisted, R. L., Pineda, O., and Tanksley, S. D. 1994. QTL analysis of trichome-mediated insect resistance in potato. Theor. Appl. Genet. 87:973-987.

Boucher, C. A., Barberis, P. A., Trigalet, A. P., and Demery, D. A. 1985. Transposon mutagenesis of *Pseudomonas solanacearum*: Isolation of Tn*5*-induced avirulent mutants. J. Gen. Microbiol. 131:2449-2457.

Broun, P., Ganal, M. W., and Tanksley, S. D. 1992 Telomeric arrays display high levels of heritable polymorphism among closely related plant varieties. Proc. Natl. Acad. SciUSA 89:1354-1357.

Buddenhagen, I., and Kelman, A. 1964 Biological and physiological aspect of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 2:203-230.

Buddenhagen, I. W., Sequeira, L., and Kelman, A. 1962 Designation of races in *Pseudomonas solanacearum*. Phytopathology 52:726.

Carney, B. F., and Denny, T. P. 1990 A cloned avirulence gene from *Pseudomonas-solanacearum* determines incompatibility on *Nicotiana tabacum* at the host species level. J. Bacteriol. 172:4836-4843.

Danesh, D., Aarons, S., Mcgill, G. E., and Young, N. D. 1994 Genetic dissection of oligogenic resistance to bacterial wilt in tomato. Mol. Plant Microbe. Interaction. 7:464-471.

Foolad, M. R., Jones, R. A., and Rodriguez, R. L. 1993 RAPD markers for constructing intraspecific tomato genetic maps. Plant Cell Rep. 12:293-297.

French, E. R., and De Lindo, L. 1982 Resistance to *Pseudomonas solanacearum* in potato: Specificity and temperature sensitivity. Phytopathology 75:1408-1412.

Gadish, I., and Zamir, D. 1987. Differential zygotic abortion in an interspecific *Lycopersicon* cross. Genome 29:156-159.

Ganal, M. W., Broun, P., and Tanksley, S. D. 1992. Genetic mapping of tandemly repeated telomeric DNA Sequences in tomato (*Lycopersicon esculentum*). Genomics 14:444-448.

Ganal, M. W., Lapitan, N. L. V., and Tanksley, S. D. 1988. A molecular and cytogenetic survey of major repeated DNA sequences in tomato (*Lycopersicon esculentum*). Mol. Gen. Genet. 213:262-268.

Gebhardt, C., and Salamini, F. 1993. Restriction fragment length polymorphism analysis of plant genomes and its application to plant breeding. Int. Rev. Cytol.. Vol. 135. 135.

Gebhardt, C., Ritter, E., Barone, A., Debener, T., Walkemeier, B., Schachtschabel U., Kaufmann, H., Thompson, R. D., Bonierbale, M. W., Ganal, M. W., Tanksley, S. D., and Salamini, F. 1991. RFLP maps of potato and their alignment with the homoeologous tomato genome. Theor. Appl. Genet. 83:49-57.

Gebhardt, C., Ritter, E., Debener, T., Schachtschabel, U., Walkemeier, B., Uhrig, H., and Salami, F. 1989. RFLP analysis and linkage mapping in *Solanum tuberosum*. Theor. Appl. Genet. 78:65-75.

Grant, M. R., Godiard, L., Straube, E., Ashfield, T., Lewald, J., Sattler, A., Innes, R. W., and Dangl, J. L. 1995. Structure of the *Arabidopsis RPM*1 gene enabling dual specificity disease resistance. Science 269:843-846.

Grimault, V., and Prior, P. 1993. Bacterial wilt resistance in tomato associated with tolerance of vascular tissues to *Pseudomonas solanacearum*. Plant Pathol. 42:589-594.

Jones, D. A., Thomas, C. M., Hammond-Kosack, K. E., Balintkurti, P. J., and Jones, J. D. G. 1994. Isolation of the tomato *Cf*-9 gene for resistance to *Cladosporium fulvum* by transposon tagging. Science 266:789-793.

Kaloshian, I., Lange, W. H., and Williamson, V. M. 1995. An aphid-resistance locus is tightly linked to the nematode-resistance gene, Mi, in tomato. Proc. Natl. Acad. Sci. USA 92:0622-625.

Kelman, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum* Tech. Bull. No. 99: NC Agric. Exp. Stn.

Kelman, A., and Sequeira, L. 1965. Root to root spread of *Pseudomonas solanacearum*. Phytopathology 55:304-309.

Kinzer, S. M., Schwager, S. J., and Mutschler, M. A. 1990. Mapping of ripening-related or -specific cDNA clones of tomato. Theor. Appl. Genet. 79:489-496.

Klein-Lankhorst, R., Rietveld, P., Machiels, B., Verkerk, R., Weide, R., Gebhardt, C., Koornneef, M., and Zabel, P. 1991. RFLP markers linked to the root knot nematode resistance gene *Mi* in tomato. Theor. Appl. Genet. 81:661-667.

Krausz, J. P., and Thurston, H. D. 1975. Breakdown of resistance to *Pseudomonas solanacearum* in tomato. Phytopathology 65:1272-1273.

Kresovich, S., Williams, J. G. K., Mcferson, J. R., Routman, E. J., and Schaal, B. A. 1992. Characterization of genetic identities and relationships of *Brassica oleracea* L. via a random amplified polymorphic DNA assay. Theor. Appl. Genet 85:190-196.

Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., and Newburg, L. 1987. Mapmaker: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

Lehmann, E. L. 1975. Nonparametrics. McGraw-Hill, New York.

Leonards-Schippers, C., Gieffers, W., Schaferpregl, R., Ritter, E., Knapp, S. J., Salamini F. and Gebhardt C. 1994. Quantitative resistance to *Phytophthora infestans* in potato—A Case Study for QTL mapping in an allogamous plant species. Genetics 137:67-77.

Li, Z. K., Pinson, S. R. M., Marchetti, M. A., Stansel, J. W., and Park, W. D. 1995. Characterization of quantitative trait loci (QTLs) in cultivated rice contributing to field resistance to sheath blight (*Rhizoctonia solani*). Theor. Appl. Genet 91:382-388.

Martin, G. B., Williams, J. G. K., and Tanksley, S. D. 1991. Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc. Natl. Acad. Sci. USA 88:2336-2340.

Mew, T. C., and Ho, W. C. 1976. Varietal resistance to bacterial wilt in tomato. Plant Dis. Rep. 60:264-268.

Mew, T. W., and Ho, W. C. 1977. Effect of soil temperature on resistance of tomato cultivars to bacterial wilt. Phytopathology 67:909-911.

Michelmore, R. W., Paran, I., and Kesseli, R. V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. SciUSA 88:9828-9832.

Miller, J. C., and Tanksley, S. D. 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. Theor. Appl. Genet. 80:437-448.

Mindrinos, M., Katagiri, F., Yu, G. L., and Ausubel, F. M. 1994. The *A. thaliana* disease resistance gene *RPS*2 encodes a protein containing a nucleotide-binding site and leucine- rich repeats. Cell 78:1089-1099.

Nakaho, K., and Takaya, S. 1993. Resistance of tomato rootstock cultivars to *Pseudomonas solanaearum* evaluated by infection rate under different testing conditions. Pages 138-141 in: Bacterial Wilt. ACIAR Proc. No. 45. G. L. Hartman and A. C. Hayward, eds. Watson Ferguson Co., Brisbane, Australia.

Panaccione, D. G., Scottcraig, J. S., Pocard, J. A., and Walton, J. D. 1992. A cyclic peptide synthetase gene required for pathogenicity of the fungus *Cochliobolus carbonum* on maize. Proc. Natl. Acad. Sci. USA 89:6590-6594.

Paterson, A. H., Lander, E. S., Hewitt, J. D., Peterson, S., Lincoln, S. E., and Tanksley, S. D. 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction length fragment polymorphisms. Nature 335:721-726.

Perera, K. D. A., Hartman, G. L., and Poulos, J. M. 1993. Inoculation procedures and the evaluation of peppers for resistance to Pseudomonas solanacearum. Pages 193-198 in: Bacterial Wilt. ACIAR Proc. No. 45. G. L. Hartman and A. C. Hayward, eds. Watson Ferguson Co., Brisbane, Australia.

Prior, P., and Steva, H. 1990. Characteristics of strains of *Pseudomonas solanacearum* from the French West Indies. Plant Dis. 74:13-17.

Prior, P., Beramis, M., Chillet, M., and Schmit, J. 1990. Preliminary studies for tomato bacterial wilt (*Pseudomonas solanacearum* E. F. Sm.) resistance mechanisms. Symbiosis 9:393-400.

Prior, P., Grimault, V., and Schmit, J. 1994. Resistance to bacteria wilt (*Pseudomonas solanacearum*) in tomato: Present status and prospects. Pages 209-223 in: Bacterial Wilt. ACIAR Proc. No. 45. G. L. Hartman and A. C. Hayward, eds. Watson Ferguson Co., Brisbane, Australia.

Reiter, R. S., Williams, J. G. K., Feldmann, K. A., Rafalski, J. A., Tingey, S. V., and Scolnik, P. A. 1992. Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc. Natl. Acad. SciUSA 89:1477-1481.

Rogowsky, P. M., Sorrels, M. E., Shepherd, K. W., and Langridge, P. 1993. Characterization of wheat-rye recombinants with RFLP and PCR probes. Theor. Appl. Genet 85:1023-1028.

Ruskortekaas, W., Smulders, M. J. M., Arens, P., and Vosman, B. 1994. Direct comparison of levels of genetic variation in tomato detected by a GACA-containing microsatellite probe and by random amplified polymorphic DNA. Genome 37:375-381.

Saiki, R., Scharf, S., Mullis, K. B., Horn, G. T., Ehrlich, H. A., and Arnheim, N. 1985. Enzymatic amplification of beta globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350-1354.

Sambrook, J., Fritsch, E. F., Maniatis, T., and Irwin, N. 1989. Molecular Cloning: A Laboratory Manual Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Sandbrink, J. M., Van Ooijen, J., Purimahua, C. C., Vrielink, M., Verkerk, R., Zabel, P., and Lindhout, P. 1995. Localization of genes for bacterial canker resistance in *Lycopersicon peruvianum* using RFLPs. Theor. Appl. Genet 90:444-450.

Schmit, J. 1978. Microscopic study of early stages of infection by *Pseudomonas solanacearum* E. F. S. on "in vitro" grown tomato seedlings. Proc. 4th Int. Conf. Plant Pathol. Bacteriol. 841-856.

Scott, J. W., Somodi, G. C., and Jones, J. B. 1993. Testing tomato genotypes and breeding for resistance to bacterial wilt in tomato. Pages 126-131 in: Bacterial Wilt. ACIAR Proc. No. 45. G. L. Hartman and A. C. Hayward, eds. Watson Ferguson Co., Brisbane, Australia.

Segal, G., Sarfatti, M., Schaffer, M. A., Ori, N., Zamir, D., and Fluhr, R. 1992. Correlation of genetic and physical structure in the region surrounding the *I2 Fusarium oxysporum* resistance locus in tomato. Mol. Gen. Genet. 231:179-185.

Sequeira, L., and Rowe, P. R. 1969. Selection and utilization of *Solanum phureja* clones with high resistance to different strains of *Pseudomonas solanacearum*. Am. Potato J. 46:451-462.

Smith, E. F. 1896. A bacterial disease of the tomato, eggplant and Irish potato (*Bacillus solanacearum* nov. sp.). U.S. Dept. Agric., Div. Veg. Phys. Pathol. Bull. 12:1-28.

Song, W.-Y., Wang, G.-L., Chen, L.-L., Kim, H.-S., Pi, L.-H., Holsten, T., Gardner, J., Wang, B., Zhai, W.-X., Zhu, L.-H., Fauquet, C.,. and Ronald, P. 1995. A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa*21. Science 270:1804-1806.

Stam, P. 1993. Construction of integrated genetic linkage maps by means of a new computer package—JoinMap. Plant J. 3:739-744.

Somodi, G. C., Jones, J. B., and Scott, J. W. 1993. Comparison of inoculation techniques for screening tomato genotypes for bacterial wilt resistance. Pages 1120-123 in: Bacterial Wilt. ACIAR Proc. No. 45. G. L. Hartman and A. C. Hayward, eds. Watson Ferguson Co., Brisbane, Australia.

Tanksley, S. D., and Loaiza-Figueroa, F. 1985. Gametophytic self-incompatibility is controlled by a single major locus on chromosome 1 in *Lycopersicon esculentum.* Proc. Natl. Acad. Sci. USA. 82:5093-5096.

Tanksley, S. D., Ganal, M. W., Prince, J. P., Devicente, M. C., Bonierbale, M. W., Broun, P., Fulton, T. M., Giovannoni, J. J., Grandillo, S., Martin, G. B., Messeguer, R., Miller, J. C., Miller, L., Paterson, A. H., Pineda,O., Roder, M. S., Wing, R. A., Wu, W., and Young, N. D. 1992. High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141-1160.

Taylor, B. H., Young, R. J., and Scheuring, C. F. 1993. Induction of a Proteinase Inhibitor II-class gene by auxin in tomato roots. Plant Mol. Biol. 23:1005-1014.

Thoquet, P., Stephens, S., and Grimsley, N. H. 1993.. Mapping of bacterial wilt resistance genes in tomato variety Hawaii7996. Page 176 in: Bacterial Wilt. ACIAR Proc. No. 45. G. L. Hartman and A. C. Hayward, eds. Watson Ferguson Co., Brisbane, Australia.

Van Ooijen, J. W., and Maliepaard, C., 1996. MapQTL (tm) version 3.0; Software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wangeningen. (In press.)

Van Ooijen, J., Sandbrink, H., Purimahua, C., Vrielink, R., Verkerk, R., Zabel, P., and Lindhout, P. 1993. Mapping quantitative genes involved in a trait assessed on an ordinal scale: A case study with bacterial canker in *Lycopersicon peruvianum.* Pages 59-74 in Molecular Biology of Tomato : fundamental advances and crop improvement. J. Yoder, ed. Technomic, Lancaster.

Vanderbeek, J. G., Pet, G., and Lindhout, P. 1994. Resistance to powdery mildew (*Oidium lycopersicum*) in *Lycopersicon hirsutum* is controlled by an incompletely dominant gene *Ol*-1 on chromosome 6. Theor. Appl. Genet 89:467-473.

Vasse, J., Frey, P., and Trigalet A. 1995. Microscopic studies of intercellular infection and protoxylem invasion of tomato rests by *Pseudomonas solanacearum.* Mol. Plant-Microbe. Interact. 8:241-251.

Vierling, R. A., and Nguyen, H. T. 1992. Use of RAPD markers to determine the genetic diversity of diploid, wheat genotypes. Theor. Appl. Genet. 84:835-838.

Vosman, B., Arens, P., Ruskortekaas, W., and Smulders, M. J. M. 1992. Identification of highly polymorphic DNA regions in tomato. Theor. Appl. Genet 85:239-244.

Whitham, S., Dineshkumar, S. P., Choi, D., Hehl, R., Corr, C., and Baker, B. 1994. The product of the tobacco mosaic virus resistance gene N: Similarity to toll and the interleukin-1 receptor. Cell 78:1101-1115.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535.

Williamson, V. M., Ho, J. Y., Wu, F. F., Miller, N., and Kaloshian, I. 1994. A PCR-based marker tightly linked to the nematode resistance gene, *Mi*, in tomato. Theor. Appl. Genet 87:757-763.

Winstead, N. N., and Kelman, A. 1952. Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. Phytopathology 42:628-634.

Yabuuchi, E., Kosako, Y., Oyaizu, H., Yano, I., Hotta, H., Hashimoto, Y., Ezaki, T., and Arakawa, M. 1992. Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Homes 1981) comb. nov. Microbiol. Immunol. 36:1251-1275.

Yabuuchi, E., Kosako, Y., Yano, I., Hotta, H., and Nishiuki, Y. 1995. Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov.: Proposal of *Ralstonia pickettii* (Ralston, Palleroni and Duodoroff 1973. comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. Microbiol. Immunol. 39:897-904.

Zamir, D., and Tadmor, Y. 1986. Unequal segregation of nuclear genes in plants. Bot. Gaz. 147:355-358.