Genetic Dissection of Oligogenic Resistance to Bacterial Wilt in Tomato

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To study resistance to bacterial wilt (caused by *Pseudomonas solanacearum*) in tomato, we analyzed 71 F_2 individuals from a cross between a resistant and a susceptible parent with 79 DNA markers. F_2 plants were inoculated by two methods: bacteria were injected into shoots of cuttings or poured into soil surrounding wounded roots. Disease responses were scored on a scale of 0 to 5. Statistical comparisons between DNA marker genotypes and disease phenotypes identified three genomic regions correlated with resistance. In plants inoculated through roots, genomic regions on chromosomes 6 and 10 were correlated with resistance. In plants inoculated through shoots, a region on chromosome 7 was significant, as were the regions on chromosomes 6 and 10. The relative impact of resistance loci on disease response differed between shoot and root inoculations. To confirm the existence of a partial resistance gene on chromosome 6, an F_2 individual homozygous for the resistant parent's alleles on chromosomes 7 and 10, but heterozygous for markers on chromosome 6, was selfed. Analysis of the F_3 progeny confirmed that a partial resistance locus was located on chromosome 6, very close to CT184. The presence of a partial resistance locus on chromosome 10 was similarly confirmed by analysis of progeny of another F_2 plant chosen on the basis of its marker phenotype.

Additional keywords: genetic mapping, host-microbe interactions, partial disease resistance, polygenic, quantitative trait loci, restriction fragment length polymorphism.

Recent advances in DNA marker technology make it possible to characterize complex genetic characters in more detail than ever before. With the use of linkage maps composed of DNA markers, genomic regions containing quantitative trait loci (QTL) can now be identified, mapped, and analyzed (Lander and Botstein 1989). DNA markers have increasingly been used to study complex agronomic traits, including fruit characters in tomato (Paterson *et al.* 1988), morphological

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differences between maize and teosinte (Doebley *et al.* 1990), hard-seededness in soybean (Keim *et al.* 1990), and seed size in *Vigna* (Fatokun *et al.* 1992). DNA markers have also been used to analyze disease resistance traits that are genetically complex. In potato, QTL associated with potato cyst nematode resistance have been characterized with restriction fragment length polymorphisms (RFLPs), and two significant associations have been found (Kreike *et al.* 1993). In common bean, resistance to bacterial blight has been examined, and four genomic regions associated with resistance have been uncovered, including one that is also associated with *Rhizobium* nodule number (Nodari *et al.* 1993). In mung bean, oligogenic resistance to powdery mildew has been studied, and three significant regions, accounting for 51% of the total variation in resistance, have been identified (Young *et al.* 1993). Finally, resistance to soybean cyst nematode has been analyzed with DNA markers, and three genomic regions have been found to be significant (Concibido *et al.* 1994), including one controlling more than 40% of the total variation.

Tomato (*Lycopersicon esculentum* Mill.) is attacked by more than 200 disease-causing organisms of diverse etiology (Jones *et al.* 1991). Bacterial wilt (BW), caused by *Pseudomonas solanacearum* (Smith) Smith, is one of the most destructive (Hayward 1991). Despite decades of interest in BW resistance, very little is known about the genetic or cellular mechanisms underlying host plant resistance (Acosta *et al.* 1964; Opeña *et al.* 1988; Tung *et al.* 1990). This is in sharp contrast to current knowledge about the genetics of pathogenicity in *P. solanacearum*, in which genes with specific biochemical and physiological roles in disease development and host range have been characterized in detail (Denny *et al.* 1990; Boucher *et al.* 1992).

Breeding BW-resistant varieties has been difficult in tomato, and attempts to develop cultivars with acceptable resistance have been unsuccessful (Acosta *et al.* 1964; Mew and Ho 1976; Opeña *et al.* 1987). One type of BW resistance in North Carolina germ plasm was found to be oligogenic and recessive (in an earlier report cited by Opeña *et al.* [1987]). In the wild tomato *L. pimpinellifolium*, resistance to BW has been found to be controlled by a small number of major genes and associated with small fruit size and indeterminate growth habit (Acosta *et al.* 1964). A study of several different BW resistance sources (Opeña *et al.* 1988) also found that only a few different resistance genes appear to be involved.

In the present study, we used DNA markers covering approximately 75% of the tomato genome to identify regions associated with partial BW resistance. These results are based on the F_2 progeny of a cross between L285, a highly resistant wild tomato (*L. esculentum* var. *cerasiforme* (Dunal) A.

Gray), and C286, a highly susceptible cultivar. Based on the DNA marker results in the F_2 generation, individuals in which only one of the putative partial resistance loci was heterozygous were chosen for further analysis in the F_3 generation.

RESULTS

Status of RFLP linkage map.

A total of 290 RFLPs were surveyed for polymorphisms between the parental genomes, but only 67 were selected for segregation analysis. Other clones were not polymorphic or consisted of minor polymorphic bands unsuitable for further study. Of the polymorphic RFLP markers analyzed, 59 mapped to 11 linkage groups on the tomato genetic map (Fig. 1). The remaining markers were unlinked at a LOD score of 3.0 (LOD is defined in the section on data analysis in Materials and Methods), although their likely map locations can be inferred from the results of Tanksley et al. (1992). Additionally, 80 oligonucleotide primers were tested as possible random amplified polymorphic DNA (RAPD) markers. Of these, only 12 produced consistent bands suitable for segregation analysis, and all 12 RAPD markers mapped to regions of the genome well-populated with RFLP loci (data not shown). Including both linked and unlinked RFLP and RAPD markers analyzed in this study, approximately 1,220 centimorgans (cM) of the tomato genome could be tested for linkage to BW resistance. This value is based on an estimate in which the intervals between all linked markers, plus a segment of 20 cM around each of the unlinked markers, were added together. This represents about 75% of the "statistical"

tomato genome, so it is possible that additional genetic factors related to BW resistance were missed.

The linkage groups in this study were generally anchored to the published tomato genetic linkage map (Tanksley et al. 1992), although a few clones mapped to different positions than those previously reported (Fig. 1). In these cases, we probably mapped duplicate loci with DNA sequences homologous to those mapped by earlier workers. Some tomato chromosomes were poorly populated with markers, especially chromosome 8 (Fig. 1). While the average level of DNA polymorphism between the parents was 23%, different chromosomes exhibited markedly different levels of DNA polymorphism. At one extreme, 10 out of 14 RFLP markers (71%) were polymorphic on chromosome 2, while only one out of 25 markers (4%) was polymorphic on chromosome 8. This unusual observation was not investigated further.

Disease responses of parents and F_1 and F_2 plants.

Two independent shoot and two root inoculation tests were performed, with two or more replicate cuttings for each F_2 individual per experiment. Tests for homogeneity (based on analysis of variance) between the two root inoculation experiments indicated no significant genotype × experiment effect ($p = 0.309$). Likewise, analysis of variance indicated no significant difference between the two shoot inoculation experiments ($p = 0.262$).

In the root inoculation experiments, the mean disease values for the resistant parent (L285), the F_1 hybrids, and the susceptible parent (CLN286) were 0.2, 0.75, and 5.0, respectively. In the shoot inoculation experiments, the mean disease
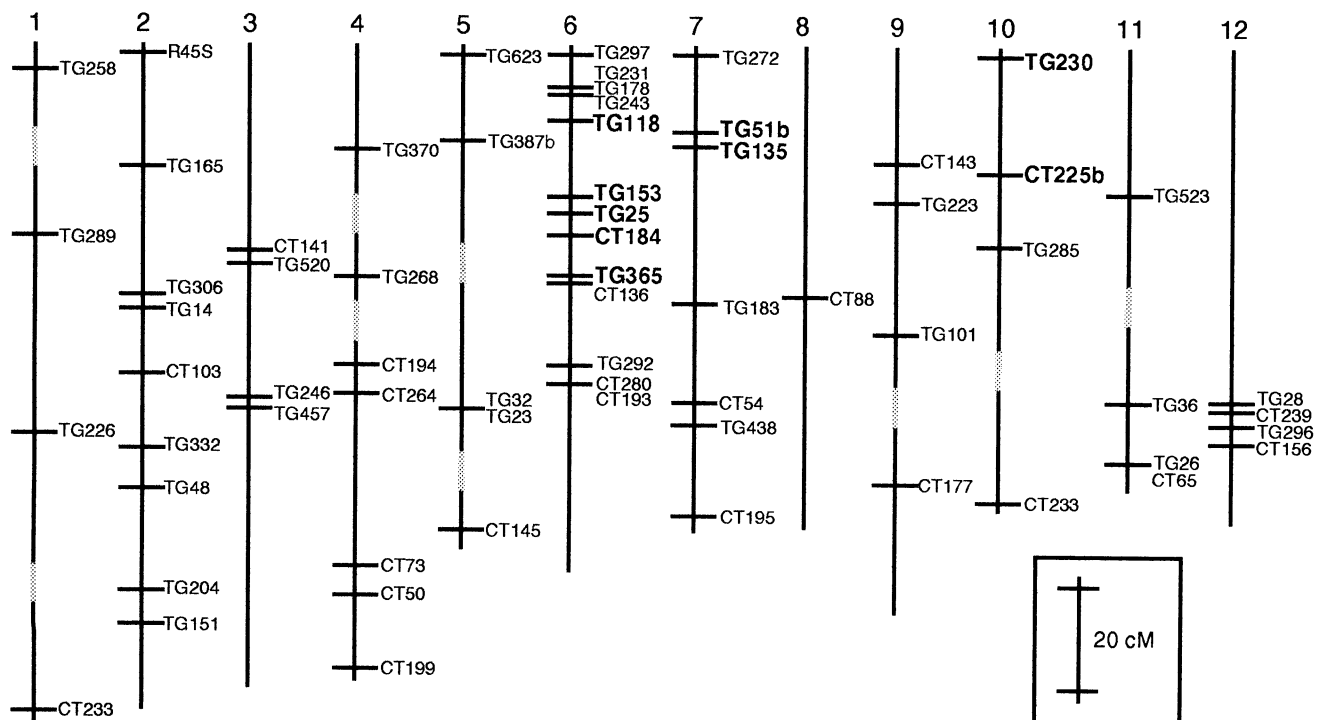

Fig. 1. Linkage map of tomato showing locations of restriction fragment length polymorphism (RFLP) markers analyzed in this study. Tomato chromosomes are shown as vertical lines, and the locations of RFLP markers are indicated by short horizontal lines. RFLP loci mapping to locations different from those reported in Tanksley et al. (1992) are followed by the letter b. Distances between markers are based on Tanksley et al. (1992). A scale in centimorgans is shown in the lower right. Intervals between markers with "likely linked" LOD scores of less than 3.0 (see Materials and Methods) are noted by a shaded segment; all other intervals had LOD scores exceeding 3.0. RFLPs discussed in the text are indicated in larger bold type.

indices of L285, F_1, and CLN286 were 0.5, 1.5, and 5.0, respectively. Thus, the resistant parent and F_1 showed a high degree of resistance independent of the inoculation method. The resistant parent and F_1 hybrids were more susceptible when inoculated through shoots than when inoculated through roots. Similarly, the mean disease response in the F_2 population was higher after shoot inoculation (2.25) than after root inoculation (1.47). In shoot inoculations, the distribution of BW response was more symmetric than in root inoculations. In the root inoculation experiments, the response was distinctly skewed toward resistance (data not shown).

RFLP markers associated with BW resistance after root inoculation.

Two genomic regions contained DNA markers that showed strong associations with BW disease response in F_2 plants inoculated through roots. One region was on chromosome 6, and the other was on chromosome 10 (Table 1 and Figs. 1 and 2). In both cases, the evidence for a partial resistance locus in these regions far exceeded the threshold (LOD > 2.4) established for this study.

On chromosome 6, RFLP marker CT184 showed the highest LOD score (Table 1 and Fig. 2). The difference in average disease score between homozygotes for the L285 allele of CT184 and homozygotes for the C286 allele was nearly three disease score units (Fig. 3). This translates to a difference in disease phenotype of virtually no symptoms in L285 homozygotes compared to obvious wilting in more than one leaf in C286 homozygotes. Heterozygotes were more resistant than the midpoint between the parents, but less resistant than L285. According to Mapmaker-QTL analysis, this region accounted for 77% of the variation in disease response.

While CT184 was most strongly associated with resistance, other RFLPs on chromosome 6 also showed an association with disease response after root inoculation, especially TG118, TG153, TG25, and TG365, which were all significant at LOD > 2.4 (Fig. 2). According to the Mapmaker-QTL analysis of the root inoculation data, the most likely location for a resistance locus was in the interval between CT184 and TG365, approximately 4 cM away from CT184 (Fig. 2).

On chromosome 10, a second genomic region near CT225b was strongly associated with resistance (Table 1 and Fig. 2). The difference in average disease phenotype between L285 homozygotes for CT225b and C286 homozygotes was less extreme than for CT184 (only two disease score units), but still quite substantial (Fig. 3). Heterozygotes for this RFLP were nearly equal to the midpoint between the parents. Ac-

cording to Mapmaker-QTL analysis, the region on chromosome 10 accounted for approximately 24% of the total variation. Together, the genomic regions on chromosomes 6 and 10 accounted for approximately 82% of the total variation in BW response.

RFLP markers correlated with BW resistance after shoot inoculation.

Three genomic regions were associated with BW response after shoot inoculation (Table 1 and Figs. 1 and 2). The regions on chromosome 6 and 10 were still associated with disease response, but so was a third genomic region on chromosome 7. Moreover, in plants inoculated through shoots the region on chromosome 10 was more strongly correlated with disease response and explained a larger portion of the variation than did the locus on chromosome 6. This is the reverse of the results in plants inoculated through roots (Figs. 2 and 3).

While Mapmaker-QTL analysis of shoot-inoculated plants indicated that the region on chromosome 6 was significantly associated with BW response (Fig. 2), its LOD score indicated that the strength of evidence for a resistance locus in this region was much lower (Table 1). The effect of this region on disease phenotype was likewise reduced in shoot-inoculated plants, compared to root-inoculated plants (Fig. 3). Still, this genomic region accounted for approximately 30% of the total variation in disease phenotype.

By contrast, the genomic region on chromosome 10 was much more strongly associated with disease response in shoot-inoculated than in root-inoculated plants (Table 1 and Figs. 2 and 3). The most likely location for a resistance locus was nearly the same (between CT225b and TG230). However, the strength of evidence for the locus on chromosome 10 and its impact on phenotype were both much greater after shoot inoculation than after root inoculation. According to Mapmaker-QTL analysis, the region on chromosome 10 accounted for approximately 38% of the variation in disease phenotype in shoot-inoculated plants.

A third genomic region, near the middle of chromosome 7, was also correlated with disease response in shoot-inoculated plants (Figs. 2 and 3). The most likely location for this locus is between RFLPs TG51b and TG135 (Fig. 2). Notably, results of root inoculation experiments did not indicate that this region is statistically associated with disease response at all (Figs. 2 and 3). Compared to the resistance loci on chromosomes 6 and 10, the one on chromosome 7 also had an unusual effect on disease phenotype. In this case, it was the al-

Table 1. Statistical results for regions of the tomato genome associated with bacterial wilt disease reaction in the F_2 generation

| Chromosome | Interval[a] | Root inoculation | | | Shoot inoculation | | |
		LOD[b]	Variation (%)[c]	d/a[d]	LOD	Variation (%)	d/a
6	CT184–TG365	17.29	77.3	0.82	4.99	30.2	1.19
7	TG51b–TG135	...[e]	...	...	4.27	24.4	−1.61
10	TG230–TG285	4.35	24.6	0.26	5.91	38.2	0.02

[a] Flanking markers of the interval in which the probability of a quantitative trait locus is greatest.
[b] Log of the odds ratio, calculated as $\log_{10}$ of the ratio between the odds in favor of a quantitative trait locus (QTL) at this location and the odds of no QTL.
[c] Percentage of total variation in bacterial wilt response explained by a quantitative trait locus in this interval.
[d] Ratio of dominance to additive effects.
[e] Not statistically significant.

lele from C286, the susceptible parent, that was associated with higher levels of resistance (Fig. 3). Approximately 24% of the total variation in disease response could be explained by the region on chromosome 7. Together, the genomic regions on chromosomes 6, 7, and 10 accounted for approximately 64% of the total variation in BW response.

F_3 progeny analysis of the putative resistance loci on chromosomes 6 and 10.

To analyze the effect of chromosome 6 on BW resistance in more detail, we examined a set of F_3 progeny in which most or all of the genetic variation was due to segregation in

this genomic region only. The DNA marker genotype data for the F_2 population indicated that plant 57 was heterozygous for several contiguous RFLPs on chromosome 6, including CT184, and was also homozygous for the L285 allele for RFLPs on chromosomes 7 and 10. Selfed progeny from this individual provided a population of plants segregating for BW resistance in which most or all of the genetic variation was due to the locus on chromosome 6.

In this F_3 population, the genomic region on chromosome 6 was still strongly associated with the resistance phenotype, with a LOD score of 9.161 at its peak, accounting for 56% of the total variation. The most likely location for a BW resistance gene was between TG25 and CT184, approximately 4 cM away from CT184. Notably, this location is on the opposite side of CT184 from that indicated in the analysis of the F_2 root inoculation results (Fig. 2).
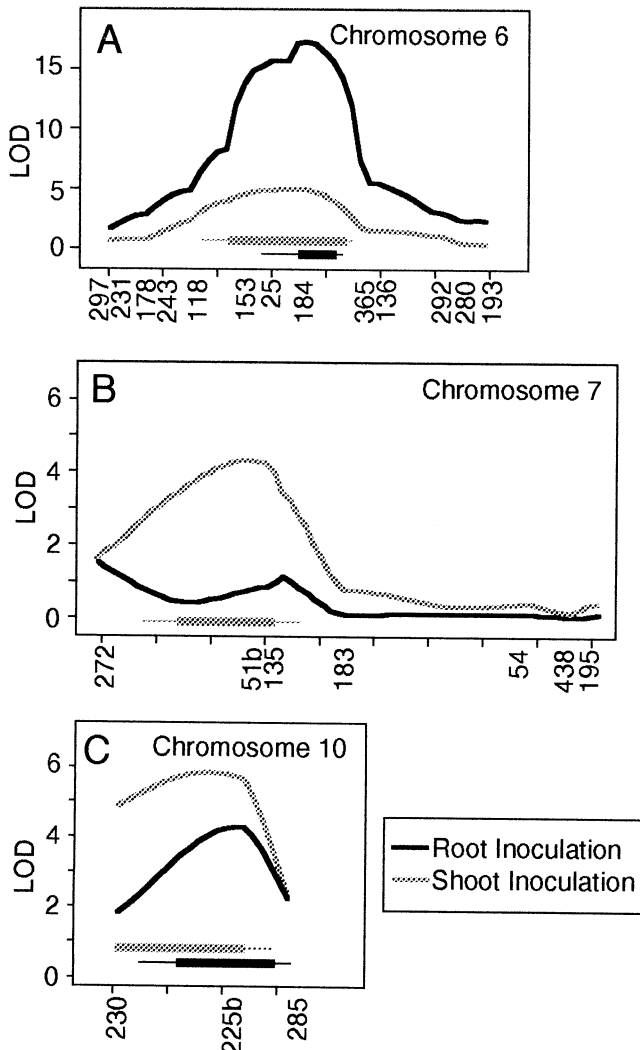

Fig. 2. Mapmaker-QTL scans of tomato chromosomes, showing statistical associations with bacterial wilt disease reaction: A, chromosome 6; B, chromosome 7; C, chromosome 10. Horizontal axes represent genetic maps of the chromosomes. The distance between tick marks is 10 cM. Vertical axes represent LOD scores for the presence of a quantitative trait locus (QTL) at the corresponding position on the chromosome. Solid lines show the results of root inoculation experiments; shaded lines show the results of shoot inoculation experiments. Near the bottom of each graph, horizontal lines indicate the most likely location for a QTL, with the thicker portion noting the chromosomal segment where the LOD score drops by 1 unit from the maximum and the thinner portion noting the segment where the LOD score drops by 2 units. Note that the vertical scale in A differs from those in B and C. Only the portion of chromosome 10 between markers TG230 and TG285 is shown.
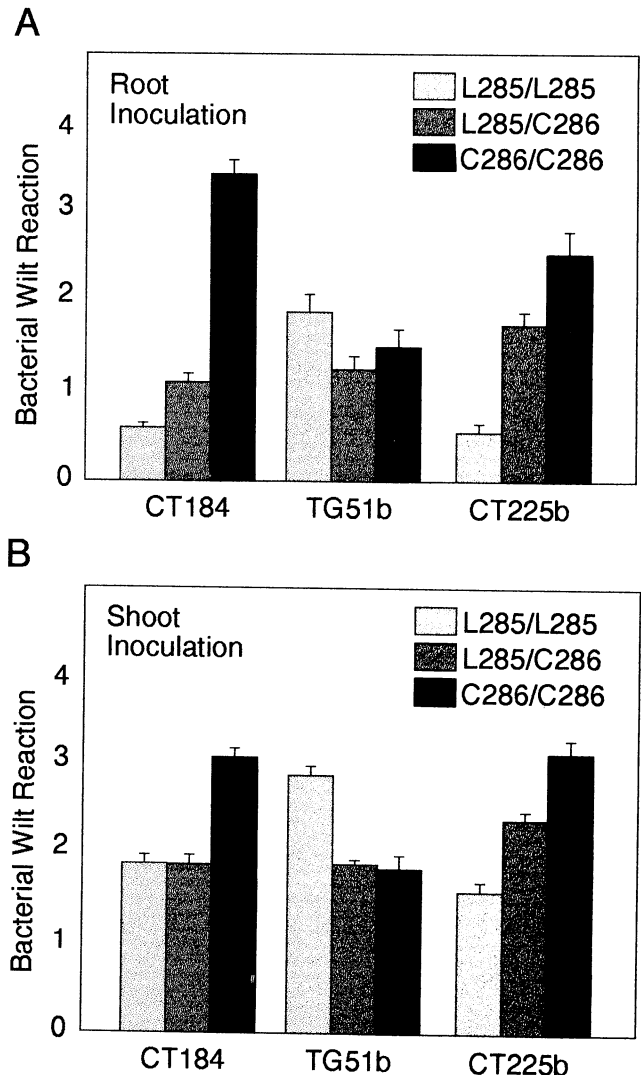

Fig. 3. Comparison of mean bacterial wilt disease reactions for different genotypic classes of restriction fragment length polymorphism (RFLP) markers CT184, TG51b, and CT225b. Average disease reaction (± standard error) for all three genotypic classes (L285/L285, L285/C286, and C286/C286) are shown. A, Root inoculation experiment. B, Shoot inoculation experiment. Results for TG51b in the root inoculation experiment are shown even though this RFLP was not significantly associated with disease reaction in this experiment.

Marker analysis of the F_2 population also indicated that plant 3 was heterozygous in the region around TG230 and CT225b on chromosome 10 but homozygous for the L285 allele on chromosomes 6 and 7. Selfed progeny from plant 3 were tested by root inoculation. The results confirmed the existence of a partial resistance locus on chromosome 10. At its peak, the LOD score for a QTL was 4.503, accounting for 70.1% of the variation. The most likely location for this resistance locus was in the interval between TG230 and CT225b, approximately 10 cM away from CT225b.

DISCUSSION

Using RFLP markers to study oligogenic BW resistance in tomato, we uncovered three genomic regions associated with disease response. By testing two different inoculation methods, we demonstrated that genomic regions are associated with resistance differentially as a function of infection site. Finally, we confirmed the importance of two of the genomic regions in BW disease response by examining F_3 populations chosen on the basis of their parents' DNA marker genotype.

Approximately 25% of the tomato genome could not be surveyed in this study because of a lack of polymorphic RFLP markers in some regions. Therefore, it is possible that additional resistance loci could be found in these parts of the genome and were missed in this study. Moreover, only 71 F_2 individuals were used in the mapping analysis, primarily because of problems in getting F_2 individuals to set fruit, which forced us to discard several plants from a larger original mapping population. Analyzing this relatively low number of individuals meant that only genomic regions with relatively large effects on disease response would be uncovered. Minor loci would probably have been missed. Finally, it is possible that multiple resistance loci could exist in the significant genomic regions uncovered. The results of Mapmaker-QTL analysis did not demonstrate whether there is just one distinct locus or, potentially, two or more closely linked resistance loci.

Although these results are based on tomato BW resistance, the use of DNA markers to examine the genetics of resistance in different organs or developmental stages can potentially be extended to any disease resistance system. This may be of general value because of the many examples of resistance that vary as a function of plant age or organ (Bartos et al. 1969; Parlevliet and Kuiper 1977). In the case of BW resistance in the L285 × C286 cross, it is clear that the locus on chromosome 6 plays a more important role in root-inoculated plants than in shoot-inoculated plants. By contrast, the locus on chromosome 10 and especially the locus on chromosome 7 play a relatively greater role in resistance in plants inoculated through shoots. With DNA markers as a basis of selection, tomato lines that carry only one of the three partial resistance genes can now be developed and examined in terms of physiology and morphology. These experiments might shed light on the role of individual partial resistance loci in the overall disease response.

The fact that the genomic regions on chromosomes 6 and 10 could be genetically dissected by the use of DNA markers provides a basis for the eventual cloning of this gene by map-based strategies (Young 1990; Arondel et al. 1992). Currently, the precise map locations of these resistance genes are

uncertain, but the results of these experiments clearly point the way toward precise mapping of the loci. In the case of the resistance locus on chromosome 6, additional DNA markers must be mapped in the region around CT184. Fortunately, numerous RFLPs have already been localized to this region (Tanksley et al. 1992), and additional markers could be added by bulked segregation analysis (Michelmore et al. 1991). Moreover, larger numbers of F_3 progeny from plant 57 could be analyzed, and those recombinant in this region (based on DNA marker genotype) could be assayed for BW disease response. Rather than being evaluated only on a 0–5 disease-scoring system, F_3 plants that are found to be recombinant in the region around CT184 could be selfed and scored for disease response in a co-dominant manner by progeny tests. Finally, using pooled groups of plants to prepare DNA samples while searching for recombinant individuals means that a very large number of progeny can be examined (Churchill et al. 1993). In this way, it should be possible to localize the partial resistance gene or genes on chromosome 6 sufficiently to carry out high-resolution genetic and physical mapping.

These experiments were carried out with only one race of P. solanacearum in two controlled environments. However, the strategy of genetically dissecting disease resistance can also be extended to studies with different P. solanacearum races in different environments. Genetic material known as recombinant inbred lines would be especially useful in these types of experiments (Burr et al. 1988). Recombinant inbreds are lines derived by single-seed descent from several individual F_2 plants down to F_6 or a later generation. In this way, a population of many genetically homogeneous lines is generated, but each line is composed of a different array of genomic blocks from the original parents. Recombinant inbred lines can be assayed for DNA marker genotype just once, and then seed from each line can be tested with many different pathogen isolates or in several different environments. In this way, the relation between genomic regions and different races of the pathogen or different environments can be examined in detail. With this in mind, we are collaborating with scientists at the Asian Vegetable Research and Development Center in Taiwan to generate recombinant inbreds from the L285 × C286 cross. These plants are now at the F_5 stage and should be ready for testing next year.

MATERIALS AND METHODS

Mapping population.

An F_2 population derived from a cross between C286 (CLN286BC1F2-25-14-7), a BW-susceptible tomato cultivar, and L285, a resistant wild tomato relative (L. esculentum var. cerasiforme), was generously provided by R. Opeña (Asian Vegetable Research and Development Center, Shanhua, Taiwan). Seventy-one F_2 individuals were grown in the greenhouse in St. Paul, Minnesota. Leaf tissue was harvested from individual F_2 plants, the parents, and an F_1 hybrid and used for DNA isolation. Simultaneously, multiple cuttings were prepared from each F_2 plant for disease assays.

Two plants in the F_2 population (plants 3 and 57) were chosen for further study on the basis of their RFLP genotypes. A cutting from each plant was grown to maturity to produce fruit, and F_3 seed was extracted. For each, 58 F_3 plants were grown in the greenhouse and processed as described above.

DNA extraction.

DNA was isolated from tomato leaves by the method of Dellaporta et al. (1983), with the exception that the extraction buffer contained 0.38% sodium bisulfite (w/v) substituted for β-mercaptoethanol.

RFLP clones.

As a source of putative RFLP markers, 161 cloned tomato genomic sequences (TG clones) and 129 cloned tomato cDNA sequences (CT clones) were generously provided by S. Tanksley (Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY). TG clones consisted of tomato sequences prepared by PstI or EcoRI digestion of genomic tomato DNA (Miller and Tanksley 1990). CT clones consisted of cDNA fragments derived from tomato epidermal tissue mRNA (Tanksley et al. 1992)

Restriction digestion and blotting.

DNA from both parents was digested with 16 restriction enzymes: AseI, BstNI, BclI, BsaBI, DraI, EcoRI, EcoRV, HaeIII, HhaI, HindIII, MspI, NdeI, NsiI, RsaI, TaqI, and XbaI. Digested DNA was resolved by 1% agarose gel electrophoresis and transferred to Hybond N+ membranes (Amersham, Arlington Heights, IL) by a method adapted from Southern (1975), with the difference being that the transfer took place in an alkaline solution of 0.1 N NaOH and 0.1 M NaCl. These blots are referred to as "parental surveys." DNA from all 71 F_2 plants was then digested with the restriction enzymes that showed the clearest fragment length polymorphism in the parental survey blots and transferred to Hybond N+ membranes to produce "F_2 blots."

DNA hybridization.

Insert sequences of RFLP clones were amplified by the polymerase chain reaction (Saiki et al. 1988), as described by Young et al. (1992). Approximately 50 ng of each insert was radiolabeled by the random hexamer reaction method (Feinberg and Vogelstein 1983). The radiolabeled product was then used to probe parental survey blots and F_2 blots as described previously (Young et al. 1992).

RAPD markers.

A total of 80 oligonucleotide primers, nine or 10 nucleotides in length (Operon Technologies, Alameda, CA), were surveyed for polymorphisms between the parents by the method of Williams et al. (1990). Polymorphic RAPDs were selected and used for segregation analysis in F_2. Amplification reactions were similar to those of Martin et al. (1991), with the following modifications: 2 ng of genomic DNA and 2 units of Taq DNA polymerase were used in a 25-µl reaction. Amplifications were performed in a Coy Tempcycler, programmed for 60 cycles of 1 min at 94° C, 1 min at 35° C, and 2 min at 72° C, followed by 7 min at 72° C on the last cycle. Reaction products were resolved by electrophoresis on 1.4% agarose gels.

Pathogenicity tests and disease scoring.

Bacterial strain UW364 was kindly provided in lyophilized form by L. Sequeira (University of Wisconsin, Madison) and used for pathogenicity testing. UW364 is a member of race 1 and biovar 4 of P. solanacearum, originally isolated from to-

mato in China (Cook et al. 1989). The isolate was retrieved by plating on tetrazolium chloride medium (Kelman 1954). Colonies with characteristic fluidal morphology of P. solanacearum were individually selected and subcultured at 30° C on tetrazolium chloride agar and casamino acids–peptone–glucose agar plates (Hendrick and Sequeira 1984). The inoculum was prepared as a shake culture in a liquid medium of casamino acids, peptone, and glucose at 30° C for 24–48 hr. Inoculum density was standardized with a Beckman DU-40 spectrophotometer coupled with dilution plates to 10^8–10^9 CFU/ml.

Plants used in inoculation experiments were rooted cuttings from F_2 plants. Tomato shoot tips were cut from each F_2 plant with a razor blade and treated with a 0.3% solution of indole-3-butyric acid. The cuttings were then planted in a mixture of vermiculite and perlite (50:50, v/v) and placed in a mist chamber for 10–14 days. They were then checked for production of lateral roots and transferred to a mixture of soil, vermiculite, and peat moss (50:25:25, v/v) and kept in the greenhouse for 3–4 days. A week before inoculation, the plants were transferred to a growth chamber with 16 hr of light (300 µE s^{-1} m^{-2}) at 30° C and 8 hr of darkness at 24° C.

For shoot inoculations, tomato cuttings were inoculated by forcing a needle into the shoot through a 15-µl drop of the bacterial suspension placed in the axil of the third expanded leaf below the shoot apex. For root inoculations, lateral roots were cut with a scalpel along one side of the plant to a depth of about 3–4 cm from the soil surface, and 50 ml of the bacterial suspension was poured over the roots. One shoot inoculation experiment was performed in a greenhouse at 28 ± 2° C; a second shoot inoculation experiment and both root inoculation experiments were performed in growth chambers with 16 hr of light (300 µE s^{-1} m^{-2}) at 30° C and 8 hr of darkness at 24° C. Two to four replicate plants of each F_2 individual plus both parents were included in each experiment. The root inoculation and shoot inoculation experiments were each repeated on two separate occasions. Four replicate cuttings of F_1 hybrids were also included in one shoot inoculation and one root inoculation experiment, both under growth chamber conditions.

After inoculation, disease readings were made at weekly intervals up to 3 wk. Data analysis (see below) was performed with readings made 2 wk after inoculation. Disease index readings were based on a scale of 0 to 5 (Winstead and Kelman 1952) as follows: 0, no symptoms; 1, mild symptoms on one leaf only; 2, obvious wilting in one or two leaves; 3, wilting in multiple leaves; 4, severe wilting throughout the plant; 5, death. The estimated disease response for each individual was the average of two separate experiments for both shoot and root inoculations (since there were no significant genotype × experiment interactions; see Results). For scoring, the plants were removed from the growth chambers, randomized, and scored for disease response without knowledge of the RFLP results or of previous disease readings.

Data analysis.

To construct a linkage map for this population, segregation data for all polymorphic DNA markers were analyzed by Mapmaker II (Lander et al. 1987). Putative linkage groups consisted of those markers showing "likely linked" LOD scores greater than 3.0. LOD is defined as the log of the odds

ratio and is calculated as $\log_{10}$ of the ratio between the odds in favor of one hypothesis (linked, in this case) and the odds in favor of an alternative hypothesis (unlinked). Marker order was based on multipoint "Compare" and "Ripple" analyses, in which the LOD score of the final order exceeded that of other orders by more than 2.0.

To identify genomic regions associated with BW resistance, all DNA marker data were analyzed with Mapmaker-QTL (Lander and Botstein 1989). To attain an experiment-wide significance level of 0.05, a LOD threshold of 2.4 was chosen. This is based on the reasoning of Lander and Botstein (1989) for a sparse marker map and a genome the size of that of tomato.

Only markers TG230, CT225b, and TG285 were scored for the F_3 progeny of F_2 plant 3, and only markers TG153, TG25, and CT184 were scored for the F_3 progeny of F_2 plant 57 (Fig. 1).

ACKNOWLEDGMENTS

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