QTLs for Tomato Powdery Mildew Resistance (*Oidium lycopersici*) in *Lycopersicon parviflorum* G1.1601 Co-localize with Two Qualitative Powdery Mildew Resistance Genes

Yuling Bai,1 Cai-Cheng Huang,2 Ron van der Hulst,1 Fien Meijer-Dekens,1 Guusje Bonnema,1 and Pim Lindhout1

1Laboratory of Plant Breeding, Graduate school for Experimental Plant Sciences, Wageningen University, Binnenhaven 5, 6709 PD Wageningen, The Netherlands; 2 Syngenta Seeds BV, Postbus 26, 1600 AA Enkhuizen, The Netherlands.


Tomato (*Lycopersicon esculentum*) is susceptible to the powdery mildew *Oidium lycopersici*, but several wild relatives such as *Lycopersicon parviflorum* G1.1601 are completely resistant. An F2 population from a cross of *Lycopersicon esculentum* cv. Moneymaker × *Lycopersicon parviflorum* G1.1601 was used to map the *O. lycopersici* resistance by using amplified fragment length polymorphism markers. The resistance was controlled by three quantitative trait loci (QTLs). *Ol-qtl1* is on chromosome 6 in the same region as the *Ol-1* locus, which is involved in a hypersensitive resistance response to *O. lycopersici*. *Ol-qtl2* and *Ol-qtl3* are located on chromosome 12, separated by 25 cM, in the vicinity of the *Lv* locus conferring resistance to another powdery mildew species, *Leveillula taurica*. The three QTLs, jointly explaining 68% of the phenotypic variation, were confirmed by testing F3 progenies. A set of polymerase chain reaction–based cleaved amplified polymorphic sequence and sequence characterized amplified region markers was generated for efficient monitoring of the target QTL genomic regions in marker assisted selection. The possible relationship between genes underlying major and partial resistance for tomato powdery mildew is discussed.

Tomato powdery mildew (*Oidium lycopersici*) has become a globally important fungus since 1986, when it was reported in The Netherlands (Paternotte 1988) and later quickly spread globally important fungus since 1986, when it was reported in


Corresponding author: Pim Lindhout; E-mail: Pim.Lindhout@WUR.NL.

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potato is based on genes having structural similarity with cloned R genes and on genes involved in the defense response. However, there are also examples of QTLs that do not overlap with the positions of known R genes, RGAs, or plant general defense genes (Geffroy et al. 2000, Qi et al. 1998, Van Berloo and Lindhout 2001). This may indicate that QTLs for resistance harbor unique resistance gene families or their regulatory loci.

The aim of our research is to investigate the genetic basis and the molecular mechanism of *O. lycopersici* resistance from *Lycopersicon parviflorum* G1.1601. In this paper, map positions of three QTLs involved in the quantitative resistance from *Lycopersicon parviflorum* are presented, and evidence is provided for co-localization of two QTLs with R genes involved in tomato powdery mildew resistance.

### RESULTS

#### Inheritance of resistance to *O. lycopersici*

from *Lycopersicon parviflorum* G1.1601.

A disease test was performed on the F2 population (*n* = 209) of *Lycopersicon esculentum* cv. Moneymaker (MM) × *Lycopersicon parviflorum* G1.1601 (G1.1601) to assess the inheritance of resistance to *O. lycopersici*. All plants were evaluated for the degree of sporulation expressed as disease index (DI) on a scale from 0 to 3. Plants of the resistant parent G1.1601 were either immune (DI = 0) or were slightly infected (DI = 1), while all plants of the susceptible parent MM were heavily infected (DI = 3) (Fig. 1). The F1 population showed a predominantly intermediate DI of 1 or 2, and the F2 plants were normally distributed over a DI range of 0 to 3, with a mean DI value of 1.8 (Fig. 1). Thus no monogenic model for the inheritance of resistance could be deduced. This result indicates that the resistance to *O. lycopersici* in G1.1601 is quantitatively inherited and is likely to be controlled by more than one gene.

### Molecular markers and map construction.

Amplified fragment length polymorphism (AFLP) analysis was performed to obtain a sufficiently large set of markers to generate a genetic linkage map from the F2 population of MM × G1.1601 (*n* = 104, discussed below). By using 14 *PstI* and 2 *MseI* primer combinations (Table 1), a total of 318 markers was obtained; 154 were MM-specific and 164 were G1.1601-specific. Initially, all markers were scored dominantly, but 34 markers could be at least partially scored codominantly, using the Quantar-Pro software (Keygene, Wageningen, The Netherlands). To improve the linkage map, 25 polymerase chain reaction (PCR)-based markers were added. These markers were mainly codominant and with known map positions on the linkage map.

<table>
<thead>
<tr>
<th>Primers/adapters</th>
<th>Sequencesa</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>MseI</em> adapter</td>
<td>5’-GACGATGAGTCTGTAGG-3’</td>
</tr>
<tr>
<td><em>M00</em> (universal primer)</td>
<td>GATGAGTCCTGAGTAA</td>
</tr>
<tr>
<td><em>MseI</em> + 1 primer M02</td>
<td>M00 + C</td>
</tr>
<tr>
<td><em>MseI</em> + 3 primers</td>
<td>M48 M00, M49 M00, M50 M00, M51 M00, M60 M00, M61 M00, M62 M00, E00 (universal primer) GACTGCGTACATGCAG</td>
</tr>
<tr>
<td><em>EcoRI</em> + 1 primer E01</td>
<td>E00 + A</td>
</tr>
<tr>
<td><em>EcoRI</em> + 3 primer</td>
<td>E35 E00, E39 E00, <em>PstI</em> adapter 3’-CTCGAGTCGTACATGCA-3’</td>
</tr>
<tr>
<td><em>P00</em> (universal primer)</td>
<td>GACTGCGTACATGCA</td>
</tr>
<tr>
<td><em>PstI</em> + 1 primer P01</td>
<td>P00 + A</td>
</tr>
<tr>
<td><em>PstI</em> + 2 primer</td>
<td>P11 P00, P14 P00, P15 P00, P18 P00, P22 P00, P00 + AA</td>
</tr>
</tbody>
</table>

a DNA sequences are always oriented from 5’ to 3’ unless indicated otherwise.

Fig. 1. Frequency distribution of the mean value of disease index (DI) for resistance to *Oidium lycopersici* in an F2 population derived from the cross *Lycopersicon esculentum* cv Moneymaker × *Lycopersicon parviflorum* G1.1601. The mean DI was an average of DI evaluated at 11, 14, and 19 days post-inoculation. The mean DI values of the two parents, the F1 population, and the overall F2 population are indicated by arrows. The population sizes are indicated between brackets.

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The table above shows the sequences of amplified fragment length polymorphism (AFLP) primers and adapters used in the study.
chromosomes 2, 4, 6, 9, 11, and 12 of a *Lycopersicon esculentum* × *Lycopersicon pennellii* map (Tanksley et al. 1992). Markers linked to the QTLs are listed in Table 2. The codominant AFLP and PCR-based markers served as bridges in map construction to merge the dominant markers into an integrated map comprising markers of both parents. Fifteen linkage groups were identified, covering a total genetic length of 761 cM. It has been reported that comigrating AFLP bands within a species are generally allele specific (Qi et al. 1998; Haanstra et al. 1999; Rouppe van der Voort et al. 1997). Therefore, 32 MM-specific AFLP markers that were in common with the markers in the genetic map published by Haanstra and associates (1999) and the locus-specific sequence characterized amplified region (SCAR) and cleaved amplified polymorphic sequence (CAPS) markers served as anchor markers to assign linkage groups to chromosomes. Consequently, 10 of the 12 chromosomes could be identified but not chromosomes 5 and 10, for which anchor markers were lacking. Clustering of markers rarely occurred in this map, since the majority of the AFLP markers were *PstI/MseI* markers that cluster less than *EcoRI/MseI* markers (Qi et al. 1998; Haanstra et al. 1999). Compared with the maps published by Tanksley and associates (1992) and Haanstra and associates (1999), the relative order of the anchor markers was consistent, and 6 of the 12 tomato chromosomes (chromosomes 1, 2, 4, 6, 11, and 12) were well saturated with markers (data not shown).

**QTL mapping.**

By applying interval mapping (IM), three QTLs for resistance to *O. lycopersici* were identified, and all resistance alleles originated from the resistant parent G1.1601. All three QTLs showed up at all infection stages (11, 14, and 19 days postinoculation [dpi]) with similar effects; thus, there was no evidence for time-dependent QTLs. One QTL, designated as *Ol-qtl1*, mapped on chromosome 6, with the highest logarithm of odds (LOD) values at CAPS marker tg25, coinciding with a genomic region containing *Ol-1* and *Ol-3* from *Lycopersicon hirsutum* (Fig. 2, Table 3) (Huang et al. 2000). The other two LOD peaks were on chromosome 12, at a distance of 25 cM from each other (Fig. 2, Table 3). To verify whether these two peaks corresponded to two linked QTLs, cofactors at the two peak positions were chosen for a multiple QTL mapping (MQM) program. Again, two clearly distinct LOD peak profiles were obtained with a similar LOD value above 3 (data not shown). Thus, MQM confirmed the presence of two linked QTLs, designated as *Ol-qtl2* and *Ol-qtl3*, on chromosome 12. *Ol-qtl2* was flanked by CAPS markers ct99 and ct129. Remarkably, the RFLP marker CT129 is also closely linked to the *Lv* locus, a major tomato resistance gene to another powdery mildew species, *Leveillula taurica* (Chunwongse et al. 1997).

**Effects of the identified QTLs on the level of resistance.**

By using codominant markers, both dominance and additive effects could be detected in this study. All three QTLs showed only additive effects (0.34, 0.42, and 0.45) and jointly explained 68% of the total phenotypic variation (Table 3). Assuming absence of epistasis and dominance, with an almost equal additive effect of each QTL resistance allele on resistance, a linear relationship between *Oidium* resistance and the number of resistance alleles at QTLs was expected. To test this hypothesis, the 104 F2 plants were grouped according to the presence of the number of putative QTL resistance alleles in these plants. A two-LOD support interval was taken as a confidence interval for the position of each QTL (Van Ooijen 1992), and markers flanking and within this region were taken as indicators for the presence or absence of one or more corresponding QTL resistance alleles. We preferably used the codominant PCR-based markers generated from RFLP markers that were closely linked to the QTLs (Table 2). In addition, two AFLP markers linked to the QTLs were converted into CAPS or SCAR markers (Table 2). Of the 104 F2 plants, 73 could clearly be genotyped without any recombination in the QTL intervals and were grouped according to the number of QTL resistance alleles. Fitting a quadratic model revealed that the quadratic term was not significant (*P* = 0.31), which indicated absence of epistatic interaction between the QTLs. An obvious linear correlation (*R*² = 0.95) was observed between increasing numbers of QTL resistance alleles and decreasing DI values

**Table 2. Primer sequences and polymerase chain reaction (PCR) conditions for the cleaved amplified polymorphic sequence (CAPS) and sequence characterized amplified region (SCAR) markers**

<table>
<thead>
<tr>
<th>Name</th>
<th>Chromosome location</th>
<th>Marker type</th>
<th>Primer sequence (5’-3’)</th>
<th>PCR annealing temperature (°C)</th>
<th>PCR product size</th>
<th>Restriction enzyme</th>
<th>Marker type</th>
<th>Marker nameb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aps1</td>
<td>6</td>
<td>CAPS</td>
<td>atgggtggtcagcatcttaag</td>
<td>56</td>
<td>1,000 bp</td>
<td><em>Sau96I</em></td>
<td>CAP</td>
<td>Aps1b</td>
</tr>
<tr>
<td>dct21</td>
<td>6</td>
<td>dCAPS</td>
<td>cagaaatagctgtcactaaca</td>
<td>50</td>
<td>185 bp</td>
<td><em>MspI</em></td>
<td>RFLP</td>
<td>CT21</td>
</tr>
<tr>
<td>tg25</td>
<td>6</td>
<td>CAPS</td>
<td>cagaaatagctgtcactaaca</td>
<td>50</td>
<td>185 bp</td>
<td><em>MspI</em></td>
<td>RFLP</td>
<td>CT21</td>
</tr>
<tr>
<td>tg111</td>
<td>6</td>
<td>dCAPS</td>
<td>cagaaatagctgtcactaaca</td>
<td>50</td>
<td>185 bp</td>
<td><em>MspI</em></td>
<td>RFLP</td>
<td>CT21</td>
</tr>
<tr>
<td>tc129</td>
<td>12</td>
<td>CAPS</td>
<td>cagaaatagctgtcactaaca</td>
<td>50</td>
<td>185 bp</td>
<td><em>MspI</em></td>
<td>RFLP</td>
<td>CT21</td>
</tr>
<tr>
<td>tc99</td>
<td>12</td>
<td>CAPS</td>
<td>cagaaatagctgtcactaaca</td>
<td>50</td>
<td>185 bp</td>
<td><em>MspI</em></td>
<td>RFLP</td>
<td>CT21</td>
</tr>
<tr>
<td>Y258</td>
<td>12</td>
<td>CAPS</td>
<td>cagaaatagctgtcactaaca</td>
<td>50</td>
<td>185 bp</td>
<td><em>MspI</em></td>
<td>RFLP</td>
<td>CT21</td>
</tr>
<tr>
<td>B432U</td>
<td>12</td>
<td>dominant SCAR</td>
<td>cagaaatagctgtcactaaca</td>
<td>50</td>
<td>185 bp</td>
<td><em>MspI</em></td>
<td>RFLP</td>
<td>CT21</td>
</tr>
<tr>
<td>tg111</td>
<td>12</td>
<td>codominant SCAR</td>
<td>cagaaatagctgtcactaaca</td>
<td>50</td>
<td>185 bp</td>
<td><em>MspI</em></td>
<td>RFLP</td>
<td>CT21</td>
</tr>
</tbody>
</table>

a The restriction fragment length polymorphism (RFLP) marker names are written with capitals (e.g., TG25), and the corresponding CAPS or SCAR marker name in lower case (e.g., tg25).

b Primers for Aps1 were published by Van Daelen (1995).
A similar linear relationship \( R^2 = 0.89 \) was observed in BC1 lines containing one to three of the QTL resistance alleles derived from different F3 plants (data not shown). If only estimated additive effects of the three QTL resistance alleles are taken into account, the predicted DI difference between the two parents would be 2.42 (Table 3), which is close to the observed DI difference of 2.66 found between the two parents (Fig. 1). This implies that the three detected QTLs accounted for nearly the complete phenotypic difference between the two parents, suggesting that most of the genetic variation is explained by these QTLs. However, hardly any F2 plants with all the QTL resistance alleles was as resistant as the resistant parent G1.1601 (Fig. 1 and Fig. 3), indicating some other minor QTLs might have escaped detection or that morphological variations among the F2 plants might complicate the evaluation of the resistance. To verify the effects of the QTLs, a disease test was performed on F3 progenies. In total, 10 F3 lines were selected that had QTL genotypes like the two parents (MM and G1.1601) or the F1 population (Table 4). As expected, segregation of resistance (DI from 0 to 3) was observed mainly in the F3 progenies from the F2 plants with a heterozygous QTL genotype. The average DI for this group was 1.2, similar to the predicted additive effects of the QTLs. The F3 progeny from F2 plants containing six QTL resistance alleles had a mean DI of 0.5, which is similar to the DI of 0.6 for the resistant parent G1.1601 in the same experiment. The F3 progeny from one F2 plant that carried six resistance alleles showed a slightly lower DI than MM. The difference between the average DI of F3 lines containing zero and six QTL resistance alleles was 2.0, which again is close to the DI difference (2.4) between the parents as controls (Table 4). This is in agreement with the results from the F2 population. In conclusion, the three QTLs jointly explained most of the resistance in the resistant parent Lycopersicon parviflorum G1.1601.

**DISCUSSION**

The disease test on the F2 population demonstrated that resistance to O. lycopersici in G1.1601 is inherited quantitatively, unlike the dominant monogenic resistance in Lycopersicon hirsutum G1.1290 and G1.1560 (Huang et al. 2000, Van der Beek et al. 1994). Three QTLs were identified that jointly explained most of the total phenotypic variation with only additive effects. In the present study, evidence has been provided that Ol-qtl2 and Ol-qtl3 are both located on chromosome 12 at a distance of 25 cM. To verify this, next progenies are generated to dissect these two QTLs by selecting recombinants between linked molecular markers. Given the limitations of QTL mapping (Van Ooijen 1992), it is hard to assume that no QTL against O. lycopersici has escaped our attention. However, the results of our study on F2 and F3 progenies clearly indicated that the three QTLs identified so far explain most of the resistance in Lycopersicon parviflorum. Since quantitative resistance is generally believed to be more durable (Johnson 1981; Lindhout 2002), it would be of great interest to combine and incorporate these QTL resistance alleles into modern tomato cultivars. Therefore, the QTL-linked PCR-based CAPS and SCAR markers that have been generated in this study are good diagnostic markers for marker-assisted breeding.

The map positions of two QTLs co-localized with the major resistance loci for tomato powdery mildews, Ol-I/Ol-3 on chromosome 6 and Lv on chromosome 12. The genetic interval of Ol-qtl1 coincided with Ol-I and Ol-3 genes that are possibly allelic and involved in HR resistance to O. lycopersici (Huang et al. 2000). Moreover, Ol-qtl2 coincided with the Lv locus, a major tomato resistance gene against Leveillula taurica (Chunwongse et al. 1997). Our observation is similar to other examples of co-localization between QTLs for resistance and major resistance genes (Caranta et al. 1997; Geffroy et al. 2000; Grube et al. 2000; Marczewski et al. 2001). The presence of both quantitative and qualitative resistance genes in the same genomic regions is not solid proof for allelism, since the accuracy of QTL mapping
does not allow pinpointing a QTL to just one gene but rather to a chromosomal region that may contain a multitude of genes. However, more evidence has recently accumulated that resistance loci tend to exist as complex loci containing clustered multigene families. For instance, the I-2 locus on chromosome 11 of tomato is involved in resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 2 and belongs to the nucleotide binding site-leucine-rich repeat (NBS-LRR) class of R genes. At this complex locus originating from *Lycopersicon pimpinellifolium*, multiple functional genes have been identified. *I2C-1* and *I2C-5* can confer partial resistance, and *I2C-K* appears to confer complete resistance specific for the *I*-2 phenotype. A similar locus conferring only intermediate resistance to *F. oxysporum* f. sp. *lycopersici* race 2 exists in the syntenic position of I-2 in the *Lycopersicon pennellii* genome (Sela-Buurlage et al. 2001). Similarly, the *Ol-qtl1* locus on chromosome 6 in the present study may correspond to another allele of the *Ol-1* locus in the *Lycopersicon hirsutum* genome. This is also in agreement with the observations that major resistance genes, once overcome by a strain of the pathogen, might conserve some residual effects. One example is that a “defeated” rice resistance gene at the *Xa4* locus acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae* (Li et al. 1999). In addition, it has been reported that modification of a monogenic resistance gene can give rise to a partial resistance gene. In *flux*, the insertion of the transposable element *Ac* in the promoter region of the *M* rust resistance gene results in partial resistance (Anderson et al. 1997). The wild species *Lycopersicon parviflorum* G1.1601 is susceptible to *Leveillula taurica* (P. Lindhout, unpublished data) and, thus, does not contain the *Lv* gene. Still, *Ol-qtl2* might be an ortholog of the *Lv* gene or a modified *Lv* gene conferring partial resistance to *O. lycopersici*.

Comparative mapping studies within the *Solanaceae* genus showed that resistance genes (both quantitative and qualitative) occurred at syntenic positions in cross-generic clusters more frequently than expected by chance, and often, clustered genes showed specificities to related and also unrelated pathogen taxa (Grube et al. 2000). The *Lv* locus belongs to one of these cross-generic clusters on chromosome 12, which, in addition to the *Lv* gene in tomato, harbors the resistance genes *Gpa2* and *Rx* in potato, conferring resistance to the potato cyst nematode *Globodera pallida* and *Potato virus X* (PVX), respectively. Intriguingly, the proteins encoded by the *Gpa2* and the *Rx1* genes share an overall homology of over 88% (amino acid identity) and belong to one class of plant resistance genes, containing a leucine zipper, nucleotide binding site, and leucine-rich repeat (Van der Vossen et al. 2000). This suggests that relatively small changes in resistance gene sequence can lead to resistance against entirely different pathogen species (Wang et al. 1998; Ellis et al. 1999). Another even more extreme example is the gene *Mi* on chromosome 6 in tomato, which renders the plant resistant to a nematode and to an aphid (Rossi et al. 1998), indicating that the identical gene sequence may be involved in resistance to very different organisms. In the present study, we mapped the *Ol-qtl2* to the cross-generic cluster containing *Lv*, *Gpa2*, and *Rx* loci. Recently, NBS homologues have been mapped to this specific genomic region in tomato (Grube et al. 2000; Pan et al. 2000; Zhang et al. 2002). Therefore, *Ol-qtl2* might be an ortholog of the *Gpa2/Rx* gene belonging to NBS homologues. To gain more knowledge about the molecular basis underlying quantitative resistance, our research aimed to clone these *Ol* genes and the QTLs and to study the resistance mechanism regulated by them. Currently, allelic tests and fine-mapping are being carried out to test our allelism hypothesis.

Table 3. The three quantitative trait loci (QTLs) associated with resistance to *Oidium lycopersici*.\(^a\)

<table>
<thead>
<tr>
<th>QTL name</th>
<th>Chromosome</th>
<th>Nearest marker</th>
<th>LOD peak value</th>
<th>Variation explained (%)</th>
<th>Additive effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ol-qtl1</em></td>
<td>6</td>
<td>tg25</td>
<td>3.8</td>
<td>16.1</td>
<td>0.34</td>
</tr>
<tr>
<td><em>Ol-qtl2</em></td>
<td>12</td>
<td>P18M51-701c</td>
<td>7.1</td>
<td>29.5</td>
<td>0.42</td>
</tr>
<tr>
<td><em>Ol-qtl3</em></td>
<td>12</td>
<td>B432u</td>
<td>5.0</td>
<td>22.3</td>
<td>0.45</td>
</tr>
</tbody>
</table>

\(^a\) Detected by interval mapping in an F\(_2\) population of *Lycopersicon esculentum* cv. Moneymaker × *Lycopersicon parviflorum* G1.1601.

**Fig. 3.** Average disease index (DI) plotted against the number of quantitative trait locus (QTL) resistance alleles present in the F\(_1\) plants of each group. The mean DI was an average of DI evaluated at 11, 14, and 19 days postinoculation. The mean DI values of the two parents, the F\(_1\) population, and the overall F\(_2\) population mean are indicated by arrows.
Upon microscopic observation, HR is reported to be the major mechanism of resistance against *O. lycopersici* in *Lycopersicon* spp. However, the resistance in *Lycopersicon parviflorum* G1.1601 was less clearly associated with HR than that in *Lycopersicon hirsutum* G1.1290 and G1.1560, suggesting that a different resistance mechanism may occur (Huang et al. 1998). *Ol-qt3* may be a good candidate for a gene that is involved in an alternative resistance mechanism different from HR, since as far as we know, it does not coincide with an *Ol* locus. Resistance mechanisms conferred by the QTLs and *Ol* loci will be characterized by gene expression studies at the molecular level and by detailed histological analysis, using near isogenic lines (NILs) that genetically differ only for presence of the QTLs or *Ol* loci. Quantitative resistance is frequently presumed to be acting in a race-nonspecific manner; thus, these QTLs will be tested with isolates from several parts of the world to check whether they confer broad-spectrum resistance. We expect that our study will result in a model in tomato that allows understanding of the potential structural relationship between genes underlying complete and partial resistance and the respective molecular mechanism.

**MATERIALS AND METHODS**

**Plant and fungus materials.**

An F2 population of 209 plants derived from an interspecific cross between individual plants of the susceptible *Lycopersicon esculentum* cv. Moneymaker and the resistant accession *Lycopersicon parviflorum* G1.1601 was used to study inheritance of the resistance. All F2 plants were selfed, but only 171 F2 plants resulted in F3 progenies with sufficient numbers of seeds.

The pathogenic fungus *O. lycopersici*, which originated from infected commercial tomato plants (Lindhout et al. 1994a), was maintained on MM plants in a greenhouse compartment at 20 ± 3°C with 70 ± 15% relative humidity (RH).

**Disease test.**

A disease test was performed by spraying one-month-old tomato plants with a suspension of 2 × 10⁴ conidia per ml. The inoculum was prepared by washing conidial spores from freshly sporulating leaves of heavily infected MM plants in tap water and was used immediately. For the disease test of the F2 population, experiments were carried out according to a randomized block design. Six blocks were used, each containing two plants of each parent and of the F1 population and 34 to 35 F2 plants. For testing the F2 lines, a complete randomized block design was used. Each of two blocks contained the 10 F1 lines (24 plants per line) and two parents (24 plants each). The inoculated plants were grown in a greenhouse at 20 ± 3°C with 30 to 70% RH.

Fungal growth was evaluated at 11, 14, and 19 dpi for the F2 population and at 14 and 18 dpi for the F3 progenies. A disease index was used where 0 = no sporulation; 1 = slight sporulation, but less than 5% foliar area affected; 2 = moderate sporulation, 5 to 30% foliar area affected; 3 = abundant sporulation, more than 30% foliar area affected.

**AFLP analysis.**

Total DNA was extracted from frozen young leaves as described by Van der Beek and associates (1992). About half (n = 104) of the F2 population was selected for AFLP analysis based on the following criteria: i) equal representation of the three disease classes (0 ≤ DI ≤ 1, 1 < DI ≤ 2, 2 < DI ≤ 3); ii) a large amount of DNA extracted per F2 plant; and iii) a large number of F2 seeds obtained. The AFLP procedure was performed as described by Vos and associates (1995), with some modifications according to Qi and Lindhout (1997). Restriction enzymes, adapters, and primers used are listed in Table 1. The following primer combinations were used: P11M48, P14M49, P14M50, P14M60, P14M61, P14M62, P15M48, P18M50, P18M51, P22M50, P22M60, E35M48, and E39M50. The underlined primer combinations have also been used for the tomato genetic map by Haanstra and associates (1999). The 5’ end of the selective Eco primer was labeled with radioactive ³²P, and the selective *Pst* primer was labeled with IRD700 or IRD800. Electrophoresis and gel analysis for ³²P-labeled AFLPs was done as described by Vos and associates (1995), and IRD-labeled AFLPs were analyzed on a LI-COR 4200 DNA sequencer, essentially following the method published by Myburg and Remington (2000).

**SCAR and CAPS analysis.**

CAPS marker *Tm2a* was used as described by Sorbir and associates (2000), and primers for CAPS *Aps1* and CP60 have been published by Van Daelen (1995) and Bendahmane and associates (1997), respectively. Other PCR-based CAPS and SCAR markers were generated from RFLP markers previously mapped by Tanksley and associates (1992). The sequences of the RFLP markers were either available as expressed sequence tags (ESTs) in the SolGenes database or were obtained by sequencing (Baseclear, Leiden, The Netherlands) bacterial clones containing the RFLP probes obtained from Cornell University, Ithaca, NY, U.S.A. Primers (Table 2) were designed by using the DNASTar software package (DNASTar, Madison, WI, U.S.A.) and were used to amplify the genomic DNA of the two parents (MM and G1.1601). If no polymorphism between the two parents was observed, the amplification products were subjected to restriction analysis with different restriction enzymes or were sequenced (Baseclear) to detect a polymorphism. Polymorphisms detected by sequencing, for which no diagnostic enzymes were available, were converted into dCAPS (derived CAPS) markers, if possible, according to the method described by Neff and associates (1998). Each PCR reaction (25 µl) contained 100 ng of genomic DNA, 1× PCR-reaction buffer, 50 ng of each forward and reverse primer, 0.2 mM dNTPs, and 0.5 unit Taq polymerase in demi water. PCR conditions were: 1 cycle at 94°C for 5 min, followed by 35 cycles of 30 s at 94°C, 30 s at annealing temperature (see Table 2), 45 s at 72°C, and a final extension of 7 min at 72°C. The PCR products were separated on 1.5% agarose gel stained with ethidium bromide, and DNA fragments were

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**Table 4. The disease index (DI) value of the tested F3 lines**

<table>
<thead>
<tr>
<th>Plant</th>
<th>No. of QTL resistance alleles in the F3 plants and the parent plants</th>
<th>No. of F3 lines tested</th>
<th>No. of plants tested</th>
<th>Average DI</th>
<th>No. of plants in three DI classes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0 ≤ DI ≤ 1</td>
</tr>
<tr>
<td>F3</td>
<td>6</td>
<td>4</td>
<td>96</td>
<td>0.5</td>
<td>80</td>
</tr>
<tr>
<td>F1</td>
<td>3 (as F1 genotype)</td>
<td>5</td>
<td>119</td>
<td>1.2</td>
<td>56</td>
</tr>
<tr>
<td>F2</td>
<td>0</td>
<td>1</td>
<td>24</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>MM</td>
<td>0</td>
<td>24</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G1.1601</td>
<td>6</td>
<td>24</td>
<td>0.6</td>
<td>20</td>
<td>4</td>
</tr>
</tbody>
</table>

* The DI average was a mean of DI evaluated at 14 and 18 days postinoculation.
visualized by UV light. About 3 µl of crude PCR product was digested in a total volume of 15 µl for 2 to 3 h with 1 unit of the appropriate restriction endonuclease. Buffers and temperature were as described by the manufacturer. After digestion, DNA fragments were separated on 2 to 3% agarose gel and visualized by UV light.

For the conversion of AFLP to CAPS and SCAR markers, 32P-labeled amplification products were excised from a dried AFLP gel and were resuspended in 50 µl H2O. The AFLP fragments were reamplified using the corresponding unlabeled AFLP primers based on standard conditions, as described above with an annealing temperature of 56°C. The PCR products were cloned using PEGM-T Easy vectors and were transformed into DH5α-competent cells. To ascertain that the proper AFLP fragment was isolated, DNA samples of four colonies for each AFLP marker were sequenced (Greenomics, Wageningen, The Netherlands). New primers internal to the AFLP selective primers were designed to amplify the genomic DNA of the two parents (MM and G1.1601). The primer design, PCR, and restriction analysis were carried out as described previously for the conversion of ESTs.

Map construction and QTL mapping.
JOINMAP 3.0 (P. Stam and J. W. Van Ooijen, CPRO-DLO, Wageningen, The Netherlands) was used to generate a genetic map applying the Kosambi’s mapping function. QTL mapping was performed using MapQTL 4.0 (J. W. Van Ooijen and C. Maliepaard, CPRO-DLO, Wageningen, The Netherlands). A LOD threshold value of 3 was set for declaring a QTL in IM (Van Ooijen 1999). After IM, a two-LOD support interval was taken as a confidence interval for a putative QTL (Van Ooijen 1992). Markers at the LOD peaks were taken as cofactors for running the MQM program to verify the results of IM.

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LITERATURE CITED


