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Quantitative Trait Loci Analysis of Powdery Mildew Disease Resistance in the *Arabidopsis thaliana* Accession Kashmir-1

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ABSTRACT

Powdery mildew diseases are economically important diseases, caused by obligate biotrophic fungi of the Erysiphales. To understand the complex inheritance of resistance to the powdery mildew disease in the model plant *Arabidopsis thaliana,* quantitative trait loci analysis was performed using a set of recombinant inbred lines derived from a cross between the resistant accession Kashmir-1 and the susceptible accession Columbia *glabrous1*. We identified and mapped three independent powdery mildew quantitative disease resistance loci, which act additively to confer disease resistance. The locus with the strongest effect on resistance was mapped to a 500-kbp interval on chromosome III.

ALTHOUGH commonly encountered, genetically complex or polygenic resistance that consists of multiple resistance loci against one pathogen race is poorly understood. This is primarily a result of the historic difficulty in studying genetically complex traits. However, with the inception of molecular DNA markers and quantitative trait locus (QTL) mapping, dissecting polygenic forms of disease resistance into component loci is now feasible (Tanksley 1993). Numerous studies to quantify and identify QTL affecting pathogen resistance in many crop species have been performed (for reviews see Michelmore 1995; Young 1996; Kover and Caicedo 2001).

The small crucifer *Arabidopsis thaliana* (L.) Heynh. provides several unique advantages for cloning and characterizing plant disease resistance genes. Arabidopsis is a host for all types of phytopathogens including bacteria, fungi, viruses, plant parasites, and nematodes (Kunkel 1996; Buell 1998). This, combined with the multitude of publicly available molecular tools, including a complete genome sequence (Arabidopsis Genome Initiative 2000), means that the cloning of disease resistance genes can proceed more quickly in Arabidopsis than in other plant species. Although there have been several QTL studies of natural variation in Arabidopsis (for a review see Alonso-Blanco and Koornneef 2000), QTL analysis has not yet been applied to pathogen resistance in this model plant species.

The obligate pathogenic fungi belonging to the Erysiphales (Ascomycetes) are the causal agents of powdery mildew diseases (Braun 1987). Among susceptible hosts are several species of economic importance, including barley, wheat, pea, grape, cabbage, and tomato, and various ornamental bushes. Resistance to powdery mildew has been studied in many plant species with both mono- and oligogenic resistance reported (Lundqvist *et al.* 1991; Lohnes and Bernard 1992; Reddy *et al.* 1994). Arabidopsis is also a host for powdery mildew with four isolates from three species reported to infect this plant (Koch and Slusarenko 1990; Adam and Somerville 1996; Xiao *et al.* 1997; Plotnikova *et al.* 1998). Mutational studies identified several Arabidopsis mutants that show significant qualitative resistance to this pathogen (Frye and Innes 1998; Vogel and Somerville 2000). A complementary approach is to study natural resistance in different accessions of Arabidopsis. At present, a minimum of eight loci controlling natural resistance to powdery mildew (designated *RPW* for *r*ecognition of *p*owdery milde*w*) have been described, including both mono- and digenic resistance conferred by semidominant or recessive disease resistance genes (Adam and Somerville 1996; Xiao *et al.* 1997). Recently two genes conferring resistance to powdery mildew were cloned from Arabidopsis, neither of which belongs to the abundant class of disease resistance genes with nucleotide binding site and leucine-rich repeat motifs (Hammond-Kosack and Jones 1997). One encodes two related small novel proteins, designated *RPW8.1* and *RPW8.2* (Xiao *et al.* 2001), and the second encodes *EDR1*, a map kinase kinase kinase (Frye *et al.* 2001).

Of the 93 Arabidopsis accessions found to be resistant to the powdery mildew *Erysiphe cichoracearum* UCSC1 (Salmon) in a previous study, Kashmir-1 (Kas-1) was highly resistant (Adam and Somerville 1996; Adam *et al.* 1999). Using QTL analysis, the genetic basis for the variation in powdery mildew resistance in a set of recom-

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binant inbred lines (RIL) derived from a cross between Kas-1 and the susceptible Arabidopsis accession Columbia *glabrous* (Col-*gl1*) was found to be complex. Here, we describe the identification and mapping of three powdery mildew quantitative resistance loci and the genetic fine mapping of the major powdery mildew resistance QTL identified in this cross.

MATERIALS AND METHODS

Plant materials: The seeds for Col-*gl1* were obtained from Dr. C. R. Somerville (Carnegie Institution of Washington). The accession Kas-1 was originally obtained from the Arabidopsis Information Service seed bank (Frankfurt, Germany) and was taken through a generation of single seed descent to enhance genetic uniformity. Seeds from a single F_1 (Col-*gl1* × Kas-1) plant of a cross, in which Col-*gl1* was used as the female, were grown and self fertilized. Three hundred F_2 plants from the (Col-*gl1* × Kas-1) cross were self fertilized and advanced to the F_6 generation by single seed descent. A random sampling of 129 RIL was chosen from the 300 for genetic analysis. Seeds for these 129 F_6 (Col-*gl1* × Kas-1) RIL are deposited at the Arabidopsis Biological Resource Center (Columbus, OH).

Plant growth procedures: Seeds were sown in commercial compost (Pro-Mix HP; Premier) in 6-inch square "jumbo square" pots (Belden Plastics) and fertilized with Peters fertilizer 20-20-20 (NPK). The pots were covered with plastic lids to ensure high humidity and incubated at 4° for 48 hr. The pots were transferred to a controlled environment with a 14-hr photoperiod (100–150 $\mu E/m^{-2}/sec^{-1}$ in the 400- to 700-nm range) at 22°. After germination, the plastic lids were removed. The plants were thinned 10–12 days after germination to give an average of 20 plants, uniformly distributed per pot. The plants were grown for ~3 weeks (until the third and fourth leaves were fully expanded) before inoculation.

Inoculation procedures: *E. cichoracearum* isolate UCSC1 was used throughout this study as a source of inoculum (Adam and Somerville 1996). Pure cultures of the isolate were created by repeated single colony subculturing. *E. cichoracearum* UCSC1 was grown and maintained on the secondary host plant squash (*Cucurbita maxima* cv Kuta; Park Seed, Greenwood, SC). Three-week-old squash plants were inoculated with the powdery mildew fungus and grown in a phytocell at 22°. Heavily infected squash leaves 10–11 days postinoculation (dpi) were used as the source of fungal inoculum. Inoculation of the Arabidopsis plants was performed by dusting conidia from squash leaves in a draft-free environment at a height of ~1 m above the plants to achieve an even distribution of conidia. Inoculation densities were >100 conidia/mm². After inoculation, plants were moved to a humidified chamber (100% relative humidity, 22°) for 1 hr to stimulate spore germination. Plants were then moved back to normal growth conditions and were evaluated for their disease reaction 7 dpi.

Evaluation of disease reaction phenotypes: The disease reaction (DR) phenotype scores were evaluated by visual inspection of the level of fungal growth observed on the adaxial surface of the inoculated third and fourth leaves of each individual plant. The scale of notation for the DR score ranged from 0, for resistant plants similar to Kas-1, to 4 for susceptible plants similar to Col-*gl1*. The DR score notation was as follows: 0, no fungal growth visible to the naked eye; 1, a limited amount of fungal hyphal growth (<25% leaf coverage) with no conidiophores; 2, an intermediate density of fungal growth (50% leaf coverage) accompanied by small restricted patches with conidiophores; 3, >75% leaf coverage by fungal growth with some conidiophores; 4, total coverage of the leaf by the fungi with abundant conidiophores conferring a "powdery" appearance.

Approximately 20 plants per RIL were grown with two Col-*gl1* plants as positive controls for inoculation in the same pot. Plants were observed for disease symptoms 7 dpi. Data were recorded only from pots in which the two control Col-*gl1* plants possessed a highly susceptible phenotype (*i.e.*, a DR score of 4). Each RIL was scored in this manner on two separate occasions. Each plant within the RIL was scored individually for fungal growth and the average DR score for ~40 plants was then used as the DR score for that RIL.

Microscopy: To ascertain whether microscopic lesions occurred on inoculated leaves, dead cells were stained with a lacto-phenol solution [250 μg/ml trypan blue in phenol, lactic acid, glycerol, and water (1:1:1:1)]. Leaves from Col-*gl1* and Kas-1 plants were collected in tubes and vacuum-infiltrated twice in the lacto-phenol solution. Then the tubes were placed in a boiling water bath for 2 min and allowed to cool for 1 hr. The leaves were destained in the 1:1:1:1 solution for 1 hr and examined under bright field illumination (Vogel and Somerville 2000). Observations of the third and fourth leaves were made at different time points from 6 hr postinoculation to 7 dpi.

The development of the fungus was assayed by the measurement of the total hyphal length of 10 colonies developing on the third and fourth leaves of both the parental lines at 1, 2, 3, and 4 dpi. This experiment was repeated three times. Very lightly inoculated leaves (~10 conidia per leaf) were harvested and cleared in 95% ethanol. Then the hyphae were stained with 250 μg/ml trypan blue in a solution of lactic acid, glycerol, and water (1:1:1) for 15 min, rinsed in the same solution, and mounted (Adam and Somerville 1996). The leaves were observed with a Leica microscope and individual colonies were photographed using a digital camera (Pixera, Los Gatos, CA). The total hyphal length per colony was measured and calculated with NIH IMAGE software (http://rsb.info.nih.gov/nih-image/).

RIL map generation: For each F_6 line, small-scale DNA preparations from 30 to 50 seedlings grown in the greenhouse were performed as described by Dellaporta *et al.* (1983). This method yielded 3–5 μg of genomic DNA per 0.5 g of fresh weight of tissue. To generate a map on the basis of the 129 F_6 (Col-*gl1* × Kas-1) RIL, codominant polymerase chain reaction (PCR)-based molecular markers that were previously mapped in crosses between Columbia and Landsberg *erecta* (Ler) were screened using DNA from the parental plants (Lister and Dean 1993; Bell and Ecker 1994). DNA markers were tested for polymorphisms and informative markers that were evenly distributed roughly every 20 cM were selected (Darvasi and Soller 1994; Young 1996). Seventeen simple sequence length polymorphism (SSLP) markers, 8 cleaved amplified polymorphic sequences (CAPS) markers, and the phenotypic marker *GLABROUS1* were used in the generation of the map (Table 1). Only one marker, CD3-69(PCR), was specifically generated for this study (Table 2). Amplification conditions for PCR were described by Bell and Ecker (1994) for SSLP markers and by Konieczny and Ausubel (1993) for CAPS markers.

The RIL map was generated from the data obtained from the 129 F_6 lines using MAPMAKER version 3.0 computer software program for the PC (Lander *et al.* 1987). The program option "RI lines obtained by selfing" was used to analyze the data. The two-point analysis command "group" (LOD = 3, maximum distance between markers = 40 cM) was first adopted to define the different linkage groups to which the molecular markers belonged. Multipoint analysis was then used with an error estimate of 1%. The best position for the unlinked markers relative to the determined order was

TABLE 1

Publicly available molecular markers polymorphic between Col-*gl1* and Kas-1 used to generate the map depicted in Figure 4

Marker	Chromosome	Type[a]	Enzyme[b]	References and sources
nga59	I	SSLP	—	Bell and Ecker (1994)
AthZFPG	I	SSLP	—	TAIR[c]
T27K12-SP6	I	SSLP	—	TAIR[c]
nga280	I	SSLP	—	Bell and Ecker (1994)
nga692	I	SSLP	—	TAIR[c]
nga1145	II	SSLP	—	TAIR[c]
THY-1	II	CAPS	*Rsa*I	TAIR[c]
nga1126	II	SSLP	—	TAIR[c]
nga168	II	SSLP	—	Bell and Ecker (1994)
90J19T7	II	CAPS	*Msp*I	TAIR[c]
nga32	III	SSLP	—	Bell and Ecker (1994)
Z30817	III	CAPS	*Bam*HI	TAIR[c]
GL1	III	CAPS	*Taq*I	Konieczny and Ausubel (1993)
T04109	III	CAPS	*Alu*I	TAIR[c]
R30025	III	CAPS	*Hin*dIII	TAIR[c]
nga6	III	SSLP	—	Bell and Ecker (1994)
nga8	IV	SSLP	—	Bell and Ecker (1994)
nga1139	IV	SSLP	—	TAIR[c]
nga1107	IV	SSLP	—	TAIR[c]
nga225	V	SSLP	—	Bell and Ecker (1994)
nga139	V	SSLP	—	Bell and Ecker (1994)
mi137	V	SSLP	—	TAIR[c]
nga129	V	SSLP	—	Bell and Ecker (1994)
LFY3	V	CAPS	*Rsa*I	Konieczny and Ausubel (1993)
m555	V	CAPS	*Acc*I	TAIR[c]

[a] Type of marker: SSLP (simple sequence length polymorphism) or CAPS (cleaved amplified polymorphic sequence).
[b] Restriction enzyme used to display the polymorphism. No digestion needed for SSLP markers.
[c] TAIR, The Arabidopsis Information Resource (http://www.arabidopsis.org/).

assessed using the command "try." To test the map for ambiguity of neighboring markers, the command "ripple" was used. Finally, the best order for the markers in each linkage group was chosen using the option "compare." The recombination frequencies were converted to map distances in centimorgans with the Kosambi function (Kosambi 1944).

Genetic fine mapping of *RPW10*: To genetically fine map the *RPW10* locus, 120 plants derived from the F_6 RIL CK48 were infected and scored for powdery mildew disease as described above. The phenotype of the individual plants was confirmed in the next generation by infecting F_7 plants derived from the selfed F_6 plants. DNA was isolated from individual F_6 plants using the small scale DNA preparation method of Edwards *et al.* (1991). New markers generated within the T04109-nga6 interval can be found in Table 2. The amplification conditions used were those of Konieczny and Ausubel (1993) for CAPS markers.

Statistical and QTL analyses: The software program MQTL (Tinker and Mather 1995a,b) version 0.98 was used to look for QTL for resistance to powdery mildew in the RIL. This program uses the least-square methods (Haley and Knott 1992) plus partial regression coefficients from background markers to control genetic variance from nontarget QTL. Only one environment and one trait, the DR score, were analyzed. The map distances between the markers generated by MAPMAKER were entered in MQTL with all markers being considered as background markers. Heterozygotes were treated as missing data points. The data were analyzed both with simple interval mapping (SIM) and simplified composite interval mapping (sCIM; Tinker and Mather 1995a,b). The thresholds for SIM and sCIM main effects were estimated by the software program with repeated "shuffling" of the whole data set using 10,000 random permutations, as advised by Churchill and Doerge (1994), with a first error rate $\alpha = 5\%$ (Beavis 1998).

Test statistic values for SIM and for sCIM were calculated every 1 cM throughout the genome. To find the precise positions of the peaks, the command "find peak" was used. For all these locations, a confidence interval was fixed where the QTL had a 95% of chance of being present. We defined this confidence interval by the "1-LOD" support interval (Lander and Botstein 1989). The total percentage of phenotypic variation (R_T^2) was estimated with the option "make estimates." R_T^2 is the variance explained by the QTL detected divided by the total phenotypic variance of the DR score (Tinker and Mather 1995b). The percentage of phenotypic variation due to individual QTL (R^2) can be related to the test statistic for SIM with this formula: $R^2 = 1 - 1/\exp(TS/n)$, with n = number of progeny (Tinker and Mather 1995b). Potential epistatic effects between a QTL and all the other points of the genome, including the other QTL, were also tested with MQTL.

The results of the QTL analysis obtained with MQTL were verified by analysis of variance (ANOVA) with the Statview 4.5 (ABACUS Concepts, Berkeley, CA) software program. Every marker was tested for the presence of a QTL using simple

TABLE 2

Informative CAPS markers berween Col-*gl1* and Kas-1 generated for this study

Marker	Enzyme	Primers	
		Forward primer	Reverse primer
CD3-69(PCR)[a]	*Nco*I	5′ TTCATAGCTCGGCGTCTGAAG 3′	5′ CCTTCACCAACTCTATAAATG 3′
CIC3D2R[b]	*Rsa*I	5′ ATCGTCTTTGAACCGGACCTT 3′	5′ ATACCAAGTGTCAATGACACC 3′
CIC3D2L[b]	*Taq*I	5′ CAATACTAGCGCAAGCTGTCA 3′	5′ TCAGCGTAGGCATAGCTTTG 3′
CIC11G6R[b]	*Sac*I	5′ TTTGGACCTTGTGCTTGCTTC 3′	5′ ATAAGGTGGACAGCGAAGTAG 3′
M005S[b]	*Hin*dIII	5′ GTGATCTTTACTTCACTAATG 3′	5′ TTTATCCTTCCCTCTCCTAG 3′
CIC8E1R[b]	—[c]	5′ GAATCAGTAACAAACATTTCTATG 3′	5′ CACCGAAGAAGAATCCATGTT 3′

[a] Publicly available (Fabri and Schaffer 1994) and mapped with the 129 F_6(Col-*gl*1 × Kas-1) RIL to the top of chromosome IV.

[b] Developed from publicly available YAC end sequence and used to fine map *RPW10* to the bottom of chromosome III.

[c] SSLP marker.

factor ANOVA ($P < 0.05$) and for its effects (R^2 = sum of squares explained by the marker divided by total sum of squares). All markers were further analyzed in pairs with a two-factor ANOVA to test for epistasis effects between every pair of markers throughout the genome and between the QTL, using the marker closest to the QTL and the rest of the markers ($P < 0.005$).

RESULTS

DR phenotypes of Col-*gl1*, Kas-1, and the RIL: Col-*gl1* is susceptible to the powdery mildew pathogen *E. cichoracearum* UCSC1 (Figure 1). Diseased plants are characterized by the development of white powdery-like fungal growth on the surface of the leaf 7 dpi. Inoculated leaves of Kas-1, however, show little or no sign of fungal growth. Necrotic and chlorotic flecks developed 5–6 dpi on infected Kas-1 leaves. No difference in germination rates of the fungus on the different accessions was observed (data not shown). Hyphal lengths per colony at 2 dpi ($P < 0.05$), 3 dpi ($P < 0.01$), and 4 dpi ($P < 0.01$) were significantly shorter on Kas-1 than Col-*gl1* (Figure 2). By 4 dpi, colonies on Kas-1 were only 45% the size of those on Col-*gl1*. The cell death observed with the naked eye in Kas-1 ∼5–6 dpi was detected as early as 9 hr postinoculation (as observed by trypan blue staining) in Kas-1 but was absent in Col-*gl1*. Cell death, however, was infrequent (<5% of infection sites) and in many cases did not appear to result in the death of the fungal colony (data not shown).

On the basis of extensive examination of the inheritance of powdery mildew resistance in F_3 (Col-*gl1* × Kas-1) lines, the inheritance of disease resistance appeared to be complex (data not shown). In agreement with this conclusion, the distribution of the DR scores for the 129 RIL showed a multimodal distribution (Figure 3) with a mean DR score of 2.2 and a standard deviation (SD) of 1.3. This distribution is significantly different from the bimodal distribution expected if resistance was conferred by a single resistance gene, indicating that the inheritance of the resistance to powdery mildew in this cross is complex.

Map construction: A total of 26 markers, consisting of 17 SSLPs, 8 CAPS, and *GL1*, were analyzed (Table 1). The segregation data of these markers were used to obtain the linkage map shown in Figure 4. The raw segregation data can be obtained upon request or can be viewed at http://carnegiedpb.stanford.edu/shauna/kas.dataprint.htm and http://www.genetics.org/supplemental/. The 26 markers were assigned to five linkage groups with a total length of 378.3 cM and an average spacing of 14.6 cM (SD = 6.1 cM). The longest distance between markers was 33.2 cM, between Z30817 and *GL1*. The genetic length of each linkage group was comparable to the lengths reported for other mapping populations and all markers were located and ordered to similar positions as on previous Arabidopsis maps (Lister and Dean 1993; Alonso-Blanco *et al.* 1998).

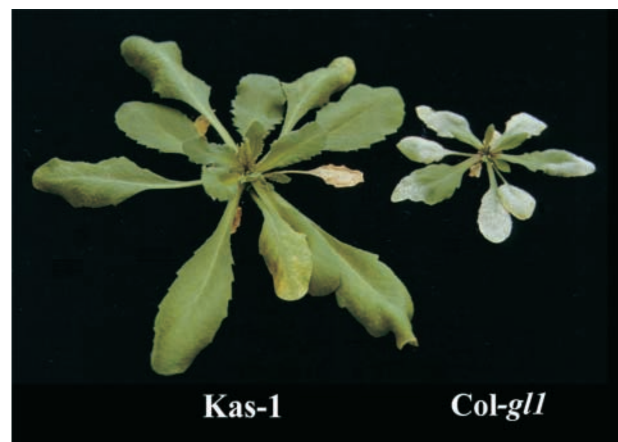

Figure 1.—Parental phenotypes Col-*gl1* and Kas-1, 7 dpi with *Erysiphe cichoracearum* UCSC1. Col-*gl1* is susceptible and is characterized by total coverage of inoculated leaves by hyphae and abundant conidiophores, whereas Kas-1 is resistant and exhibits no fungal growth, although necrotic flecks, which develop late (5–6 dpi), can be observed.

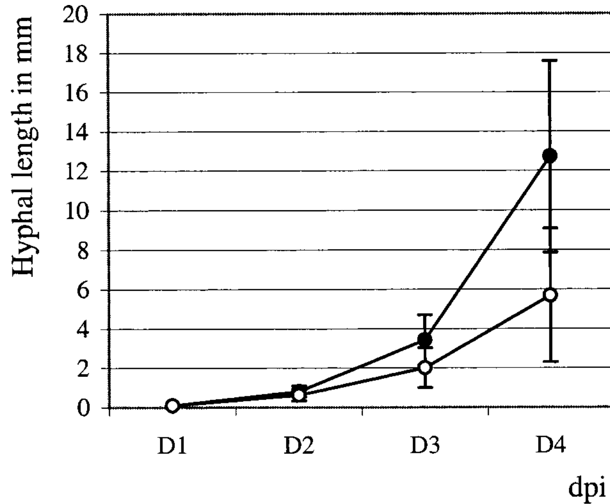

Figure 2.—Hyphal length. Total hyphal length of individual powdery mildew colonies measured on Col-*gl1* (solid circles) and Kas-1 leaves (open circles) 1, 2, 3, and 4 dpi. Each data point corresponds to the average hyphal length per colony ($n = 30$) with the SD.

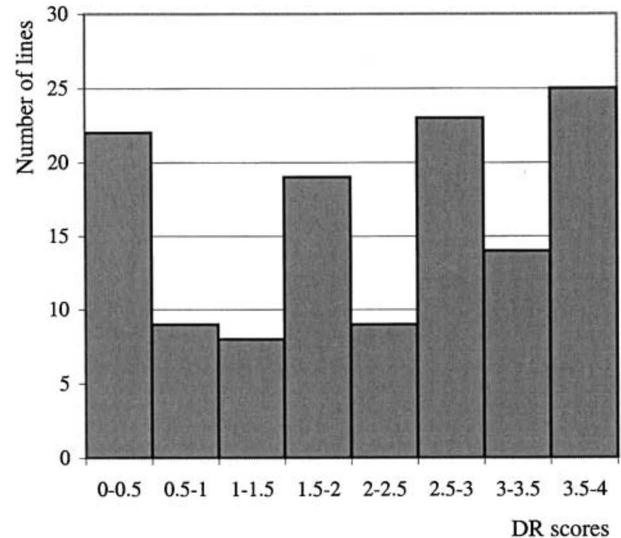

Figure 3.—Frequency distribution of DR scores of the 129 F_6 (Col-*gl1* × Kas-1) RIL. The DR score presented for each RIL is the average of the DR scores observed on ~40 plants. The parental DR scores are 0 for Kas-1 (resistant) and 4 for Col-*gl1* (susceptible).

Analysis of the RIL: The segregation ratio of the two homozygous classes at each marker was tested for the 1:1 expected proportion and the markers significantly distorted in their segregation ratios are indicated in Figure 4. It is clear that the segregation distortion occurred for a large portion of the genome. The single largest distortion of the segregation ratio was observed for nga8 (top of chromosome IV), where Col-*gl1* alleles were 5.1-fold more abundant than Kas-1 alleles in the 129 RIL. The remaining distortions were of the ratio of 1:2.7 or lower.

RIL are expected to be essentially homozygous by the F_6 generation with the theoretical chance of a heterozygous RIL for a given marker being 3.1%. Analysis of the segregation data revealed a much higher rate of 9.1%, indicating that some bias for the selection of heterozygous plants via the method of single seed descent had occurred.

QTL analysis: The software program MQTL was used to analyze the segregation data for the DR scores and molecular markers (Tinker and Mather 1995a,b). Graphs of the test statistics for SIM and sCIM main are shown in Figure 5. The thresholds computed were 10.3 for SIM and 26.7 for sCIM.

Three unlinked regions in the Arabidopsis genome were found to have test statistic values for both SIM and sCIM higher than the corresponding thresholds and so were designated as a QTL. Resistance alleles for all powdery mildew resistance loci were derived from the resistant parent Kas-1. *RPW10* was mapped on the bottom of chromosome III to a confidence interval of only 6 cM. The second QTL, designated *RPW11*, occurred near the marker nga139 on the top of chromosome V with a confidence interval spanning 12 cM. The region spanning the lower part of chromosome II exceeded the SIM threshold. However, the curve representing the test statistic for sCIM refined the position of this QTL to one peak centered near nga1126. The confidence interval in which the QTL lies was localized to an 11-cM region (Lander and Botstein 1989). This QTL was designated as *RPW12* (Table 3). To further confirm the authenticity of the QTL identified by the program MQTL, the entire data set was reanalyzed by one-factor ANOVA. All three QTL were confirmed by ANOVA at the 95% confidence level (data not shown).

The percentage of the total phenotypic variation (R_T^2) explained by the three QTL was calculated at 63.0% (Table 3). *RPW10* accounted for 45.0% of the total variation, *RPW11* for 17.6%, and *RPW12* for 10.4% (Table 3). The percentages of the total phenotypic variation explained by the individual QTL using ANOVA were similar to those obtained using MQTL (data not shown). Substitution of *RPW10* susceptibility alleles for resistance alleles had the greatest impact on the DR score (*i.e.*, 1.7 DR score points explained) compared to *RPW11* and *RPW12* (Table 3).

No epistatic effects were demonstrated by either MQTL or ANOVA. Therefore, all three QTL identified are additive in their effects on powdery mildew resistance.

Fine mapping of *RPW10*: As previously mentioned the number of heterozygotes observed in the F_6 (Col-*gl1* × Kas-1) RIL was approximately three times higher than expected. During scoring for powdery mildew resistance, a few RIL were observed to segregate for disease resistance in a simple Mendelian fashion. Examination of the genetic profile of several of these lines revealed

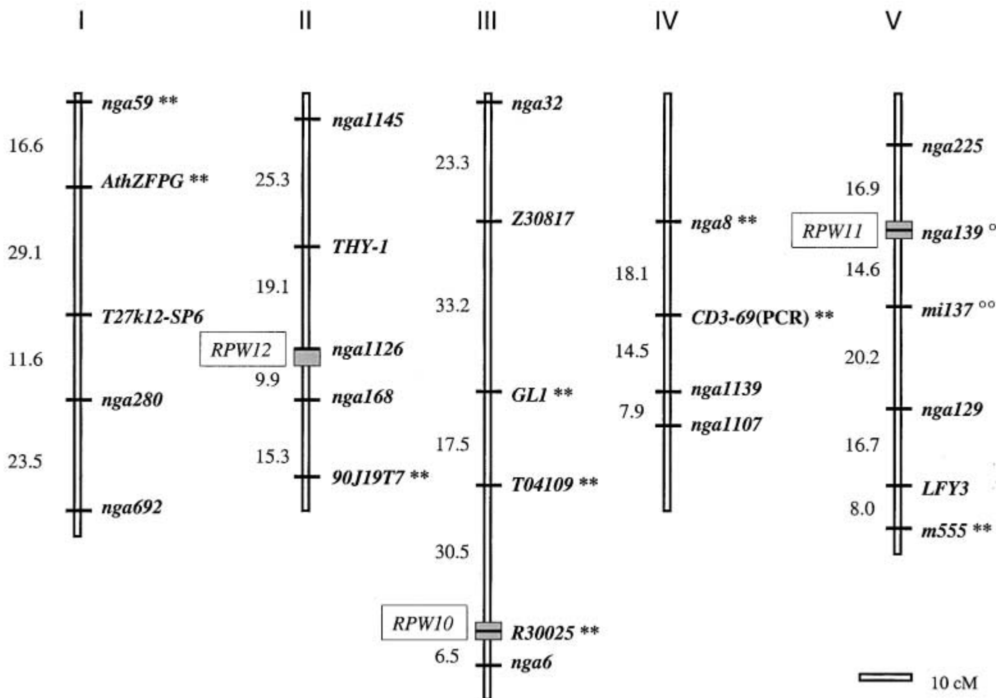

Figure 4.—Genetic map of the Col-*gl1* × Kas-1 cross. Twenty-five molecular markers (17 SSLP and 8 CAPS) and one morphological marker were mapped in 129 RIL and the map was generated with MAPMAKER (Lander *et al.* 1987). The distances are displayed in centimorgans. The placement of the first and the last marker on every chromosome was done according to the distances available from the F_8 (Ler × Col) RI map (Lister and Dean 1993). Significant deviations from the expected 1:1 distribution of Kas-1 and Col-*gl1* alleles at each marker are indicated as follows: **, Col-*gl1* alleles in excess ($P < 0.01$); ° °, $P < 0.01$; and °, $P < 0.05$, Kas-1 alleles in excess. Positions of the *RPW* loci are in boxes.

that they were heterozygous for markers on the lower arm of chromosome III, including the marker R30025, which is near *RPW10*. As the line CK48 was homozygous for the Col-*gl1* alleles at both *RPW11* and *RPW12*, this line was likely to be segregating for only the *RPW10* QTL. To confirm that this was the case, 120 plants from the F_6 line CK48 were planted and scored for powdery mildew resistance. Both resistant (DR score = 1) and susceptible plants (DR score = 4) as well as an intermediate class of plants (DR score = 2 or 3) were observed. These 120 plants were selfed and the resistance phenotypes of these plants confirmed in the F_7 generation. The segregation results obtained were compatible with a segregation ratio of resistant homozygote:heterozygote:susceptible homozygote of 1:2:1 (CK48: $\chi^2 = 2.2$, $0.5 < P < 0.25$). Thus, in CK48, resistance is conferred

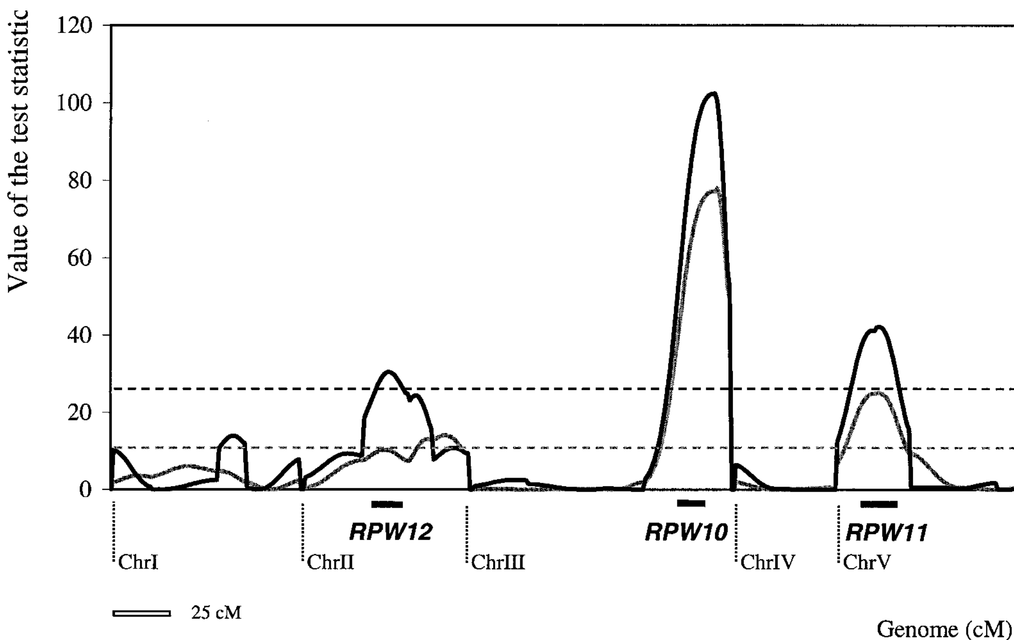

Figure 5.—QTL likelihood map for resistance to powdery mildew in the F_6 (Col-*gl1* × Kas-1) RIL. The abscissa corresponds to the genetic map in centimorgans; the limits of each chromosome are indicated by vertical dotted lines (ChrI to ChrV). The ordinal corresponds to the test statistics for SIM (simple interval mapping) and sCIM (simplified composite interval mapping) main effects generated by MQTL (Tinker and Mather 1995a). The test statistics are represented by a gray solid line for SIM and a black solid line for sCIM. The grey broken line indicates the treshold for SIM test statistics and black broken line indicates the threshold for sCIM test statistics. Three QTL were detected: *RPW12* on chromosome II, *RPW10* on chromosome III and *RPW11* on chromosome V. The location of each QTL is symbolized by a horizontal black bar (▬), as determined form the "1-LOD" support interval (Lander and Botstein 1989).

TABLE 3

Characteristics for QTL for resistance to powdery mildew identified in a Col-*gl1* × Kas-1 cross

QTL	Position of the QTL[a]	Confidence interval[b]	TS SIM[c]	LOD[d]	DR score explained[e]	Variation explained[f] (%)
RPW10	R30025 + 0 cM	−4 < R30025 < +2	77.2	17.0	1.7	45.0[g]
RPW11	nga139 ± 0 cM	−6 < nga139 < +6	4.9	5.5	0.9	17.6[g]
RPW12	nga1126 − 6 cM	−13 < nga1126 < +2	14.1	3.1	0.9	10.4[g]
						Total explained: 63.0[h]

[a] Position of the QTL as estimated by MQTL (displayed by the command "find peaks").
[b] Confidence interval, in centimorgans, for the location of the QTL (calculated with the "1-LOD" support interval).
[c] Test statistic value for SIM at the QTL location (displayed by the command "find peaks").
[d] LOD score of likelihood for the existence of a QTL, with LOD = TS × 0.22.
[e] DR score points explained by each QTL (displayed by the command "make estimates").
[f] Percentage of the phenotypic variation explained, as determined by MQTL.
[g] One QTL at a time, using the formula $R^2 = 1 - 1/\exp(TS/n)$ with TS SIM and $n = 129$, the number of progeny.
[h] All three QTL (estimated by TQTL with the command "make estimates").

by a single locus with the resistance allele being semidominant. To further define the location of *RPW10,* 120 CK48 lines were genotyped using the CAPS marker R30025 and the SSLP marker nga6. The marker T04109 was homozygous in line CK48. Results from these markers indicated the *RPW10* locus is located between R30025 and T04109. The location of this locus was further refined using codominant PCR-based markers (Table 2). The *RPW10* locus was genetically mapped to a 4-cM interval defined by the markers M005-S and CIC8-E1RE, which is ~500 kbp in size (Figure 6).

DISCUSSION

In this study, we demonstrated that powdery mildew resistance in the Arabidopsis accession Kas-1 is inherited in a polygenic fashion. Using QTL analysis of a set of RIL, three unlinked resistance QTL were identified and, for each QTL, the resistance alleles were derived from Kas-1. The QTL, designated *RPW10, RPW11,* and *RPW12,* were found to act additively to confer resistance. The demonstration that *RPW10* is allelic to the recently cloned gene *RPW8* provides additional confirmation of the validity of *RPW10* (Xiao *et al.* 2001). A genetic model based on all three QTL explained 63% of the total variation in powdery mildew resistance observed. The values for the percentage of variation explained by individual QTL and by the combined three-QTL model from these studies compare favorably to the values recorded in other studies dealing with QTL for pathogen resistance. For example, the percentage of total phenotypic variation explained ranged from 14 to 81%, with a mean of ~50% in studies summarized in Young (1996).

The inheritance of powdery mildew resistance in the accession Kas-1 was studied preliminarily by Adam and Somerville (1996). It was reported that resistance to *E. cichoracearum* UCSC1 was conferred by a single locus, designated *RPW1,* based on a population of F_2 plants and genetic mapping data from a relatively small number ($n = 54$) of F_3 lines derived from a cross between Col-*gl1* and Kas-1. One possible explanation for the discrepancy between the former and current studies is that Arabidopsis plants in the study by Adam and Somerville (1996) were inoculated with a 10-fold lower density (5–10 conidia per mm^2), which often led to patchy infections and made the scoring of disease symptoms difficult. Also, because the F_2 (Col-*gl1* × Kas-1) population seemed to fit a simple model of resistance, DR

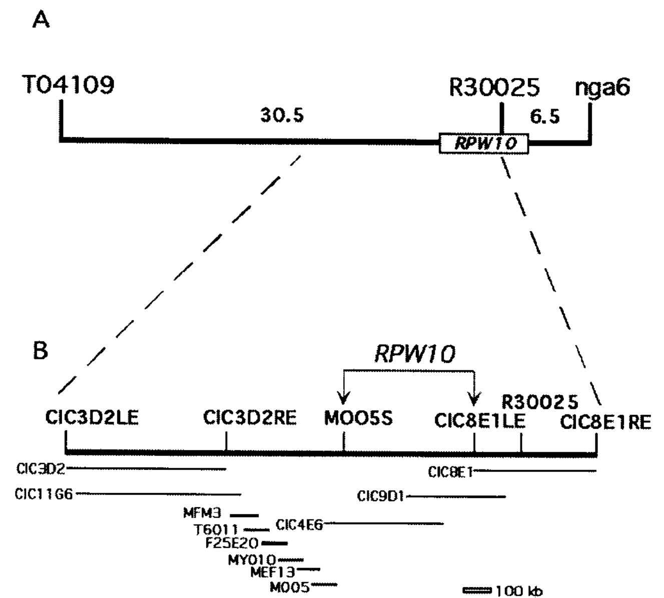

Figure 6.—Genetic mapping of the *RPW10* locus. (A) The 37-cM region of chromosome III identified by QTL analysis as containing the powdery mildew disease resistance locus *RPW10.* The confidence interval for *RPW10,* using the "1-LOD" support interval (Lander and Botstein 1989), is denoted by an open rectangle. (B) The location of *RPW10* as determined by genetic mapping. A physical map of the region was constructed from published bacterial artificial chromosome and yeast artificial chromosome physical maps (Camilleri *et al.* 1998; Sato *et al.* 1998).

scores derived from the F_3 families were rigidly categorized into three genotypic classes (*i.e.*, homozygous resistant, homozygous susceptible, or heterozygous) to reflect a single gene model. These two factors, combined possibly with the segregation distortion observed for the bottom of chromosome II, resulted in the incorrect assignment of the powdery mildew resistance genotype for a number of F_3 families, which led to an incorrect understanding of the inheritance of powdery mildew resistance. Due to the different inoculation conditions and populations used in these two studies, all resistance QTL identified in this study were given new *RPW* designations. However, we cannot exclude the possibility that *RPW1* may correspond to one of the *RPW* loci identified in this study.

The set of F_6 (Col-*gl1* × Kas-1) RIL represents the seventh set of Arabidopsis RIL generated and the fourth set that has been characterized in detail genetically (Reiter *et al.* 1992; Lister and Dean 1993; Holub and Beynon 1997; Alonso-Blanco *et al.* 1998; Deslandes *et al.* 1998; C. L. Schiff, I. W. Wilson and S. C. Somerville, personal communication). Although significant distortions in the segregation ratios of markers were detected in large portions of the genome, the magnitude and extent of the distortions were comparable to those observed for the other genetically characterized RIL (Reiter *et al.* 1992; Lister and Dean 1993; Alonso-Blanco *et al.* 1998; C. L. Schiff, I. W. Wilson and S. C. Somerville, personal communication). For the telomeric region of the lower arm of chromosome II, the distortion is present in three sets of RIL. The origin and possible sources of this segregation distortion in the F_6 (Col-*gl1* × Kas-1) RIL are presently unknown and present an interesting avenue for understanding complex gene interactions. The threefold higher level of heterozygosity observed in our set of RIL may be due to some bias for the involuntary selection of heterozygous plants during single seed descent and is probably a reflection of heterosis (Mitchell-Olds 1995).

Although most Arabidopsis accessions possess a relatively short life cycle, creating RIL is still a lengthy and laborious process, especially with Kas-1, as this accession requires a vernalization treatment to flower. However, the RIL offer several advantages, such as the possibility of permanently propagating the population without further genotyping and the advantage of studying a trait on several sibling plants per line to minimize the environmental variation (Alonso-Blanco and Koornneef 2000). Overall, the 129 F_6 (Col-*gl1* × Kas-1) RIL constructed in this study will provide useful material for the analysis of other traits that vary between these two accessions (*e.g.*, size, vernalization).

Another major application for RIL is to use the residual heterozygosity of some lines for map-based cloning of QTL using standard map-based cloning methods (Tuinstra *et al.* 1997; Lukowitz *et al.* 2000). In this study, using a single RIL (CK48) that segregated for powdery mildew resistance in a Mendelian fashion, we were able to genetically map the *RPW10* locus to an interval of ~500 kbp. This region agrees well with the confidence interval calculated by MQTL, confirming the accuracy of our QTL analysis.

The value of QTL analysis in Arabidopsis lies in the ability of this system to address fundamental questions concerning the nature of QTL for disease resistance. Disease resistance QTL have been variously proposed to be weak alleles of race-specific disease resistance genes or to be a class distinct from known disease resistance genes (*e.g.*, genes encoding elements of signal transduction pathways or genes encoding defense response components; Pflieger *et al.* 1999). In this regard, the demonstration that *RPW10* is allelic to *RPW7*, which confers resistance to *E. cruciferarum* (Junnel), lends support to the hypothesis that this locus encodes a broad-spectrum resistance mechanism (Xiao *et al.* 1997, 2001). The other feature that distinguishes *RPW10* from typical disease resistance genes is that *RPW10*-mediated resistance does not lead to the arrest of fungal growth at a specific step in the infection nor is this resistance associated with a typical rapid-acting hypersensitive necrosis response. These observations together with the novel nature of the cloned *RPW8* genes (allelic to *RPW10* and *RPW7*) support the hypothesis that resistance QTL are distinct from classical race-specific resistance genes (Xiao *et al.* 2001). Additional studies of natural sources of resistance will likely yield new insights into the nature of powdery mildew resistance.

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