QTL analysis of quantitative resistance to *Phytophthora infestans* (late blight) in tomato and comparisons with potato

Douglas J. Brouwer, Elizabeth S. Jones, and Dina A. St. Clair

Abstract: Quantitative trait loci (QTLs) for resistance to *Phytophthora infestans* (late blight) were mapped in tomato. Reciprocal backcross populations derived from cultivated *Lycopersicon esculentum* × wild *Lycopersicon hirsutum* (BC-E, backcross to *L. esculentum*; BC-H, backcross to *L. hirsutum*) were phenotyped in three types of replicated disease assays (detached-leaflet, whole-plant, and field). Linkage maps were constructed for each BC population with RFLPs. Resistance QTLs were identified on all 12 tomato chromosomes using composite interval mapping. Six QTLs in BC-E (*lb1a*, *lb2a*, *lb3*, *lb4*, *lb5b*, and *lb11b*) and two QTLs in BC-H (*lb5ab* and *lb6ab*) were most consistently detected in replicated experiments or across assay methods. *Lycopersicon hirsutum* alleles conferred resistance at all QTLs except *lb2a*. Resistance QTLs coincided with QTLs for inoculum droplet dispersal on leaves, a trait in *L. hirsutum* that may contribute to resistance, and dispersal was mainly associated with leaf resistance. Some *P. infestans* resistance QTLs detected in tomato coincided with chromosomal locations of previously mapped R genes and QTLs for resistance to *P. infestans* in potato, suggesting functional conservation of resistance within the Solanaceae.

Key words: late blight, tomato, *Lycopersicon hirsutum*, QTL mapping, disease resistance, potato.

Résumé : Des QTL (locus quantitatifs) conférant la résistance au *Phytophthora infestans* (mildiou) ont été cartographiés chez la tomate. Des populations backcross réciproques, dérivées d'un hybride entre le *Lycopersicon esculentum* cultivé et le *L. hirsutum* sauvage (BC-E, backcross avec *L. esculentum*; BC-H, backcross avec *L. hirsutum*), ont été phénotypées en utilisant trois techniques d'inoculation (foliole détachée, plante entière, au champ). Des cartes génétiques ont été produites pour chaque population BC avec des marqueurs RFLP. Des QTL de résistance ont été identifiés sur l'ensemble des douze chromosomes en pratiquant une cartographie composite d'intervalles. Six QTL dans la population BC-E (*lb1a*, *lb2a*, *lb3*, *lb4*, *lb5b* et *lb11b*) et deux QTL dans la population BC-H (*lb5ab* et *lb6ab*) ont été décelés avec la plus grande constance d'une réplication à une autre et pour l'ensemble des méthodes d'inoculation. Les allèles de résistance provenaient du *L. hirsutum* pour tous les QTL sauf *lb2a*. Les QTL pour la résistance correspondaient à des QTL contrôlant la dispersion des gouttelettes d'inoculum sur les feuilles, un caractère du *L. hirsutum* qui pourrait contribuer à la résistance, et une telle dispersion était associée à une résistance foliaire. Certains QTL de résistance au *P. infestans* détectés chez la tomate sont situés à des positions chromosomiques semblables à celles de gènes R et de QTL pour la résistance au mildiou chez la pomme de terre. Ceci suggère une conservation fonctionnelle de la résistance au sein des solanacées.

Mots clés : mildiou, tomate, *Lycopersicon hirsutum*, cartographie QTL, résistance aux maladies, pomme de terre.

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Introduction

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, can be a devastating disease of two closely related cultivated Solanaceae, tomato (*Lycopersicon esculentum* L.) and potato (*Solanum tuberosum* L. subsp. *tuberosum*), and frequently causes major crop losses worldwide (Fry and Goodwin 1997). In the U.S., the threat of late blight epidemics has been heightened by the migration of new, aggressive, clonal lineages of *P. infestans* from Mexico (Fry and Goodwin 1997). These new lineages are resistant to the fungicide metalaxyl and some are capable of infecting both tomato and potato (Goodwin et al. 1995, 1996). *Phytophthora infestans* may be controlled by fungicides, but increased environmental regulations and the spread of fungicide-resistant isolates (Goodwin et al. 1996) are re-

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stricting the available chemical control options. For these reasons, finding and using effective, long-lasting genetic resistance to *P. infestans* is increasingly important (Wastie 1991).

Resistance to *P. infestans* can be classified as either qualitative or quantitative (Thurston 1971; Wastie 1991). Race-specific resistance, also referred to as isolate-specific, is conferred by "R" genes that often exhibit qualitative inheritance. In potato, at least 11 R genes have been characterized to date, and all are monogenic dominant and qualitatively inherited (Ewing et al. 2000; Gebhardt and Valkonen 2001; Kuhl et al. 2001; Naess et al. 2001). In tomato, three isolate-specific R genes have been reported: *Ph1*, *Ph2*, and *Ph3*. *Ph1* is completely dominant, whereas *Ph2* and *Ph3* are incompletely dominant (Conover and Walter 1953; Gallegly 1960; Turkensteen 1973; Chunwongse et al. 1998; Moreau et al. 1998). Quantitative resistance, also referred to as partial resistance, tends to be multigenic and quantitatively inherited (Wastie 1991; Umaerus and Umaerus 1994). Although quantitative resistance to *P. infestans* has been established in potato (Colon et al. 1995; Collins et al. 1999), to our knowledge, it has not been documented in tomato.

Over 100 years of work on the genetics and breeding of resistance to *P. infestans* in potato has generated a wealth of information on this host–pathogen system (Thurston 1971; Wastie 1991; Umaerus and Umaerus 1994). R genes introgressed from wild potato species were first used in potato breeding programs in the 1930s. Unfortunately, new *P. infestans* isolates capable of overcoming R genes in potato cultivars evolved rapidly, limiting the usefulness of such genes for late blight control (Thurston 1971; Wastie 1991; Umaerus and Umaerus 1994; Collins et al. 1999). Similarly, *Ph*1 and *Ph2* in tomato were overcome by new *P. infestans* isolates (Conover and Walter 1953; Gallegly 1960; Black et al. 1996). The durability of the *Ph3* gene is untested, since it is currently being introgressed from *Lycopersicon pimpinellifolium* into cultivated tomato (Kim and Mutschler 2001). Modern potato breeding programs concentrate on selecting for quantitative resistance to *P. infestans* (Thurston 1971; Wastie 1991; Umaerus and Umaerus 1994; Collins et al. 1999). High levels of quantitative resistance to *P. infestans* have been bred into some potato cultivars and provided resistance over many years of exposure to *P. infestans* in the field (Thurston 1971; Wastie 1991; Turkensteen 1993; Colon et al. 1995).

The genetic basis of quantitative resistance can be studied using molecular markers and phenotypic information to map quantitative trait loci (QTLs) in segregating populations (Young 1996; Doerge 2002). QTL mapping can be a particularly powerful tool for genetic dissection of quantitatively inherited traits with low heritability whose expression is strongly influenced by the environment (Mackay 2001; Doerge 2002). QTLs controlling quantitative resistance to pathogens in several plant species have been identified (e.g., Wang et al. 1994; Caranta et al. 1997; Qi et al. 1999). In potato, QTLs for quantitative resistance to late blight have been mapped in several studies, with multiple genetic factors being detected (Leonards-Schippers et al. 1994; Meyer et al. 1998; Collins et al. 1999; Oberhagemann et al. 1999; Ewing et al. 2000; Sandbrink et al. 2000). No similar studies have been reported in tomato, a Solanaceous species whose genome is highly syntenic with potato (Tanksley et al. 1992; Grube et al. 2000).

Quantitative resistance to *P. infestans* can be difficult to assess, since environmental, developmental, and physiological factors (e.g., plant maturity, vigor, leaflet age) can influence the observed phenotype (Thurston 1971; Wastie 1991; Turkensteen 1993; Collins et al. 1999). Disease assays conducted in growth chambers or greenhouses with detached leaflets or whole plants are employed to help control environmental conditions and permit efficient testing of large numbers of genotypes (Wastie 1991). However, these assays do not measure all components of plant resistance as expressed in the field and may not be suitable for the detection of quantitative resistance (Wastie 1991; Collins et al. 1999). QTL studies can be used to determine whether resistance QTLs detected in growth chamber or greenhouse assays are also effective in conferring resistance under field conditions.

The limited genetic diversity in cultivated tomato (*L. esculentum*) has led to frequent use of wild *Lycopersicon* species as a source of genes controlling various agriculturally important traits, like disease resistance, for genetic improvement efforts (Rick and Yoder 1988). Wild *L. pimpinellifolium* is the original source of R genes *Ph1*, *Ph2*, and *Ph3* (Gallegly 1960; Chunwongse et al. 1998). Resistance to late blight has been observed in wild *Lycopersicon hirsutum* (Lobo and Navarro 1987). More recently, a single plant selection (MD1) from the self-incompatible, highly heterogeneous *L. hirsutum* accession LA2099 exhibited resistance in detached-leaflet assays to numerous *P. infestans* isolates (M. Coffey, personal communication) and was highly resistant in the field in North Carolina (R. Gardner, personal communication). Phenotypic distributions for resistance to *P. infestans* in interspecific F_1 progeny from *L. esculentum* × *L. hirsutum* LA2099-MD1 were continuous (data not shown), suggesting that resistance could be quantitative in nature. Collectively, these observations imply that LA2099-MD1 might be a novel and useful source of genes for resistance to *P. infestans*.

In this study, we have used composite interval mapping and RFLP markers to map QTLs for quantitative resistance to *P. infestans* in reciprocal *L. esculentum* × *L. hirsutum* LA 2099-MD1 backcross populations. These populations were tested in replicated detached-leaflet, whole-plant, and field disease assays. Resistance QTLs were mapped on all 12 chromosomes, indicating the multigenic nature of resistance in MD1. The use of reciprocal backcross populations permitted the detection of QTL alleles with dominant effects. Several QTLs were detected consistently across replicated experiments and assays. Resistance QTLs coincided with QTLs for inoculum droplet dispersal on the leaflet surface, a trait in *L. hirsutum* that may contribute to *P. infestans* resistance. Some resistance QTLs detected in tomato coincided with the chromosomal locations of previously mapped *P. infestans* resistance QTLs and R genes in potato, suggesting functional conservation within the Solanaceae.

Materials and methods

Plant material and maintenance

Two reciprocal backcross populations were developed

Table 1. Replicated disease phenotype assays and experiments conducted to assess the resistance to *P. infestans* in reciprocal backcross populations derived from *L. esculentum* × *L. hirsutum*.

Assay type[a]	Experiment	Isolate	Inoculum concentration[b]	Phenotype traits[c]	No. of individuals[d] BC-E	BC-H
DL	*A*	7629	1×10^3	SP, DD	213	133
DL	*B*	7629	1×10^4	SP, DD	213	133
DL	*C*	7629	1×10^4	SP	174	122
DL	*D*	7629	1×10^4	SP, DD	84	122
DL	*E*	7629	1×10^4	SP, DD	84	122
DL	*F*	7629	1×10^4	SP, DD	84	108
WP	*G*	7629	1×10^3	IS, ST	84	96
WP	*H*	7629	1×10^3	IS, ST	95	96
WP	*I*	7629	1×10^3	IS, ST	95	96
FLD	*J*	9175	1×10^3	PPI, PI, ST	73	89
FLD	*K*	Natural	—	PPI, PI, ST	73	89

Note: Experiment abbreviations used throughout the paper include assay type, experiment, and the disease phenotype trait evaluated. For example, whole plant assay *H* evaluated by stem score was abbreviated WP*H*, ST.

[a]DL, detached leaflet assay; WP, whole plant assay; FLD, field assay.

[b]Inoculum concentration measured in spores/mL.

[c]Disease phenotype traits were the length of the sporulating lesion (SP) in millimetres for detached leaflet assays; plant infection score (IS) and stem score (ST) for whole plant assays; and the percentage of the plot infected on the first date (PPI), the percentage of the plant infected in a 30-cm^3 area on the second date (PI), and stem score (ST) on second date for field assays. Five detached leaflet assays (*A, B, D, E, F*) were also scored for inoculum droplet dispersal after 24 h (DD). See Materials and methods for more details.

[d]Number of progeny plants analyzed in each experiment for reciprocal backcross populations derived from *L. esculentum* 'NC84173' × *L. hirsutum* LA2099-MD1 (BC-E, backcross to *L. esculentum*; BC-H, backcross to *L. hirsutum*).

from late blight susceptible *L. esculentum* "NC84173" and the late blight resistant genotype MD1. MD1 was a single plant selected from the wild *L. hirsutum* f. *glabratum* accession LA2099. LA2099 is highly heterozygous, self-incompatible, unilaterally incompatible with *L. esculentum*, and has inedible small green fruit (C.M. Rick, Tomato Genetic Resource Center, University of California at Davis, http://tgrc.ucdavis.edu). MD1 was identified as resistant to multiple *P. infestans* isolates in detached-leaflet assays (M. Coffey, personal communication). Seed for NC84173, a homozygous, large, red-fruited tomato breeding line (Gardner 1992), was obtained from R. Gardner, North Carolina State University. NC84173 is determinate (*sp*/*sp*) and LA2099-MD1 is indeterminate (*Sp*/*Sp*). Determinate (compact) growth habit is controlled by the *sp* (self-pruning) locus on chromosome 6 (Pnueli et al. 1998). NC84173 is hereafter referred to as E, and LA2099-MD1 as H.

Thirty-five interspecific F_1 hybrids, derived by crossing E × H and using H as the male parent, were screened using detached-leaflet assays (see Phenotyping section). First backcross (BC_1) populations were generated by crossing the most resistant F_1 as the male parent to E (BC-E, 213 individuals obtained), but as the female parent to H, owing to unilateral incongruity (BC-H, 133 individuals). All plants were maintained in a greenhouse at U.C. Davis in 4-L pots using standard horticultural practices. Material for replicated disease assays was generated by clonally propagating plants, as needed, using stem cuttings. Plants were lost over time owing to greenhouse diseases and propagation difficulties; consequently, the number of BC individuals in each experiment varied (Table 1).

Phytophthora infestans isolates

Two isolates were used in resistance assays: 7629 (A1 mating type, metalaxyl-sensitive, US-6 genotype) and 9175 (A1 mating type, metalaxyl-resistant, US-11 genotype). The isolates were collected in California and provided by M. Coffey, U.C. Riverside. US genotype designations are according to Fry and Goodwin (1997). The US-6 and US-11 lineages are genetically diverse and represent two of the four clonal lineages commonly pathogenic on tomato (Fry and Goodwin 1997). Isolates 7629 and 9175 are virulent on tomato genotypes containing *Ph1* and *Ph2*. In addition to these two isolates, the plants in the field assays were also exposed to naturally occurring *P. infestans*.

Cultures of *P. infestans* were grown at 17 °C on Rye B agar (Caten and Jinks 1968) and transferred monthly. Isolates were periodically passaged on leaves of susceptible parent NC84173 to maintain pathogenicity and profuse sporulation. Inoculum for disease assays was prepared by washing 8-day-old sporulating lesions with sterile distilled water. Spore concentrations were determined using a hemacytometer and adjusted to the desired concentration (Table 1).

Phenotyping

Late blight resistance of BC-E and BC-H was evaluated in 11 replicated experiments conducted over two years (1996

and 1997): six detached-leaflet experiments (designated *A*–*F*), three whole-plant experiments (*G*–*I*), and two field experiments (*J* and *K*) (see Table 1). Inoculum droplet dispersal on the leaflet surface was assessed in five detached-leaflet experiments. Developmental traits were assessed on plants grown in both the greenhouse and the field.

Detached-leaflet (DL) assay

Each of six DL assays included leaves from the parents (E and H), the F_1, and healthy BC-E and BC-H individuals. In each experiment, each genotype was replicated over time with three (*A*, *B*, *C*) or two (*D*, *E*, *F*) replicates and three (*A*, *B*, *C*) or four (*D*, *E*, *F*) leaflets per replicate. A randomized complete block design (RCBD) was used, considering replicates over time as blocks. Leaflets were taken from 8- to 11- (*A* and *B*), 20- to 25- (*C*), 7- to 8- (*D*), 13- to 15- (*E*), and 21- to 23-week-old (*F*) plants. Experiments *A* and *B* compared inoculum concentration (Table 1). Variation between replicated experiments was reduced using 1×10^4 spores/mL, which was used in all subsequent assays.

Tomato leaflets were clipped off greenhouse-grown plants at the petiole and placed in transparent lidded plastic boxes lined with towels moistened with sterile water. The lower surface of leaflets was inoculated with 20 μL of sporangial suspension at 1×10^3 or 1×10^4 spores/mL (see Table 1). Boxes were sealed in plastic reclosable bags to maintain high humidity and placed in a growth cabinet at 16 °C with no light. After 24 h, the inoculum droplet was removed from the leaflet using a twisted tissue wick to prevent bacterial infection that could interfere with late blight assessment. Boxes were subsequently incubated at 16 °C with 12 h light : 12 h dark. To reduce light intensity and prevent leaflet wilting, boxes were covered with two layers of mesh shade cloth (Easy Gardner, Waco, Tex.). Late blight resistance was assessed 6 days after inoculation as the length of the sporulating lesion (SP) in millimetres.

Inoculum droplet dispersal

In preliminary detached-leaflet assays, it was observed that the inoculum droplet rapidly dispersed on leaflets of the resistant parent H, and sometimes completely disappeared. In comparison, the susceptible parent E and *L. esculentum* 'T5', another susceptible control, showed no droplet dispersal. Dispersal was also observed on leaves of the F_1, although dispersal was less rapid, complete, and consistent than on H. Droplet dispersal (DD) was segregating in the backcross populations. In five detached-leaflet screens (see Table 1), DD was assessed 24 h after inoculation using a scale of 0–3 (0, no dispersal; 1, partial dispersal; 2, complete dispersal but some inoculum still visible; and 3, no surface inoculum remaining).

Whole-plant (WP) assays in growth cabinets

In three WP experiments (*G*, *H*, *I*), each genotype was tested in three replicates in time and with two cuttings per replicate in an RCBD. Cuttings of BC-E and BC-H were placed in separate flats. Each flat included up to 35 BC genotypes and parental and F_1 controls. In pilot experiments, increased inoculum resulted in better phenotypic separation of the generally more resistant individuals in the BC-H population (although both BC populations contained resistant individuals; see Fig. 1). Thus, each replicate for each backcross (three flats) was spray inoculated separately when cuttings were 8 to 10 weeks old with a total of 300 (BC-E) or 600 mL (BC-H) of a 1×10^3 spores/mL suspension of isolate 7629. After inoculation, flats were placed in a dew chamber (Conviron, Winnipeg, Man.) with an air temperature of 17–18 °C. After 24 h, the flats were moved to a growth cabinet with 12 h light (18 °C) : 12 h dark (16 °C). Spray nozzles pulsed mist every 10 s to maximize humidity. Disease was rated 7 d after inoculation using two criteria: percent plant infection score (IS) on a scale of 0–7 (0, no visible sign of disease; 1, ≤5% foliage infected; 2, 5%–25% infected; 3, >25%–50%; 4, >50%–80%; 5, >80%–95%; 6, >95%–99%; and 7, 100%) and stem resistance score (ST) on a scale of 0–4 (0, no stem lesions; 1, thread-like lesions; 2, spreading, coalesced lesions ≤2 cm; 3, lesions >2 cm; and 4, complete stem necrosis).

Field (FLD) assays

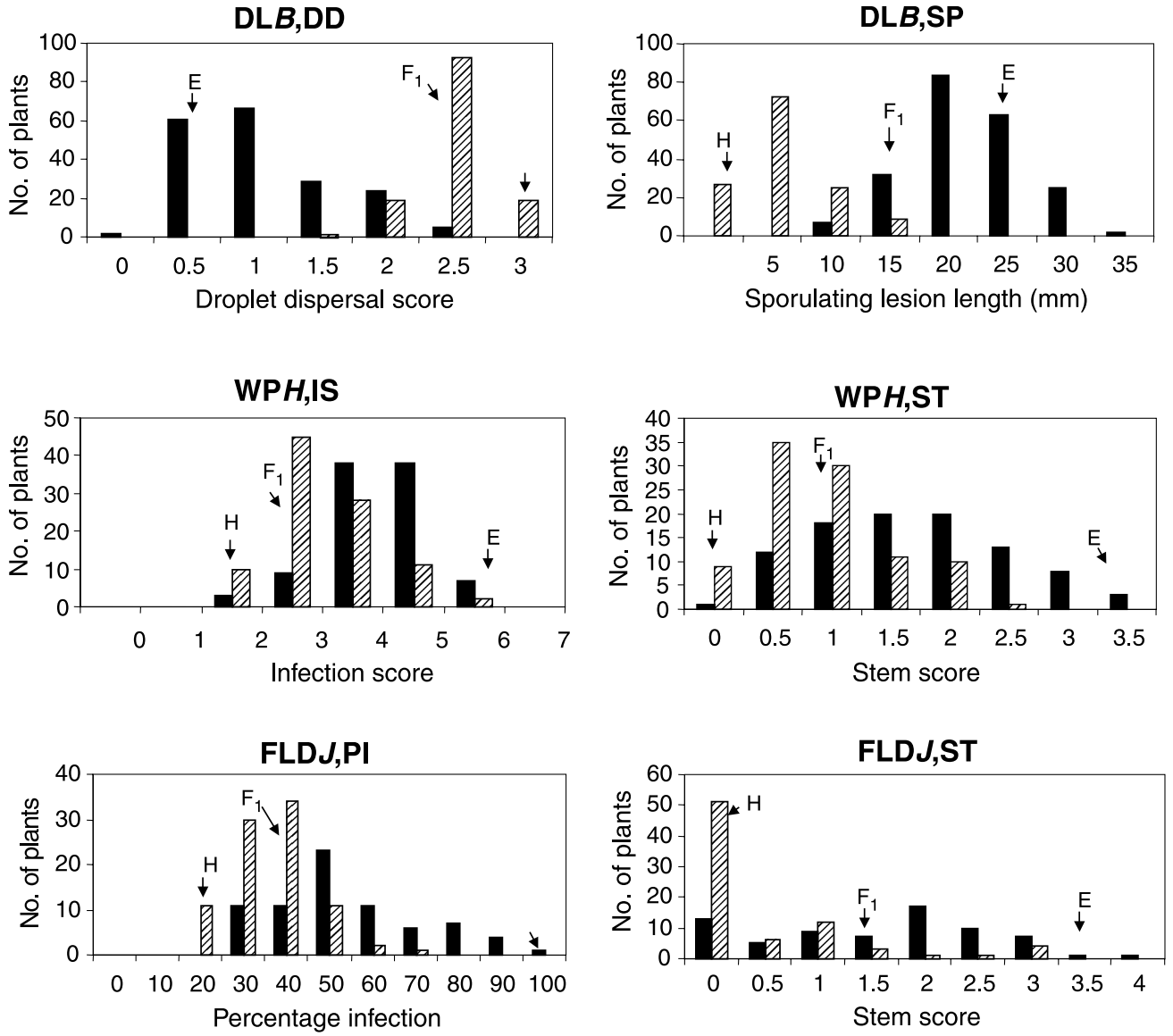
Field assays (*J* or *K*) were carried out at two northern California locations, San Juan Bautista and King City, respectively (Table 1). At each location, a RCBD with three replicates was used, and each replicate (block) included one plot of each BC genotype, eight plots of each parent, the F_1, and the susceptible cultivar *L. esculentum* 'T5'. Six-week-old cuttings, generated from greenhouse-grown plants, were transplanted to the field on 30 May (*J*) and on 5 June 1997 (*K*). Each plot contained 5 plants spaced at 0.61-m intervals, with 1.52 m between plots within a row and 3.04 m between rows. Standard horticultural practices were used, except no fungicides were applied and plants were irrigated as needed.

Assay *J* was inoculated twice, 12 and 13 weeks after transplanting, with 40 L of a 1×10^3 spores/mL sporangial suspension of local isolate 9175. Each plot was inoculated at two points within the canopy. The field was sprinkler irrigated to increase humidity before inoculation, and weekly thereafter, to encourage *P. infestans* infection and spread. Assay *J* was assessed on two dates, 16 and 21 weeks after transplanting. On the first date, disease was assessed as percentage plot infected (PPI), but this trait appeared to be associated with determinate growth and plant height, traits that were segregating in BC-E. On the second date, disease was rated as percent infection within 30 cm^3 of the inoculated site (PI) to remove any relationship with plant size, and stem resistance (ST) was rated on a scale of 0–4 (0, no stem lesions, 1, ≤20%; 2, >20%–40%; 3, >40%–60%; and 4, 60% stems with lesions). Assay *K* relied on natural infection of *P. infestans*. PPI (scored on the lower 0.5 m of foliage) was assessed 13 weeks after transplanting, and PI and ST 16 weeks after transplanting.

Plant developmental traits

In the greenhouse, plants were assessed for the number of nodes 10 weeks after sowing, and determinate growth 22 weeks after sowing as the mean of three assessments for the number of nodes between each inflorescence (Pnueli et al. 1998). At field site *J*, plants were scored for fruit set as the presence or absence of fruit 21 weeks after transplanting. At field site *K*, plants were scored for fruit set and plant height (cm) 14 weeks after transplanting.

Fig. 1. Representative phenotypic trait distributions for selected experiments *B*, *H*, and *J*. Histograms are labeled using the experiment and trait abbreviations used in Table 1. Assay types were DL, detached-leaflet assay; WP, whole-plant assay; FLD, field assay. Disease phenotype traits were the length of the sporulating lesion (SP) in millimetres for detached leaflet assays, plant infection score (IS) and stem score (ST) for whole plant assays, and the percentage of the plot infected on the first date (PPI), the percentage of the plant infected in a 30-cm^3 area on the second date (PI), and stem score (ST) on the second date for field assays. Means for NC84173 (E), LA2099-MD1 (H), and the F_1 are indicated with arrows. Black and hatched boxes denote BC-E and BC-H progeny, respectively.



Data analysis

Analysis of variance (ANOVA) within each backcross population was performed on each experiment using the plot means and PROC GLM of SAS (SAS Institute Inc. 1996). Genotypes and replications were considered random effects. Broad sense heritabilities were calculated as $H^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$ using the mean squares from the SAS GLM procedure where $\sigma_g^2 = (MS_{genotype} - MS_{rep \times genotype})$. Normality was checked with PROC UNIVARIATE using the NORMAL option in SAS. Phenotypic correlations among traits were obtained separately for each backcross population using genotypic means across replications and were calculated using Microsoft Excel 2000. Trait data for experiments within detached-leaflet and whole-plant assays were tested for homogeneity of variance using Bartlett's test (Steel et al. 1997) and pooled when homogeneous.

Map construction

DNA isolation, restriction enzyme digestion, and Southern analysis were performed as described previously (Williams and St. Clair 1993; Truco et al. 2000). Genomic (TG) and cDNA (CD and CT) clones were kindly supplied by S. Tanksley (Cornell University, Ithaca, N.Y.) and were mapped previously in tomato (Pillen et al. 1996; Tanksley et al. 1992). Clones were selected at 10- to 20-cM intervals from the map of Tanksley et al. (1992) to represent the entire tomato genome. Codominant polymorphisms between E and H were identified by digesting parental DNA with five restric-

tion enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I) and probing with clones. The RFLPs were scored for 104 and 98 probe–enzyme combinations on 213 BC-E and 133 BC-H individuals, respectively. Linkage maps were constructed for each backcross separately using MAPMAKER/EXP 3.0 (Lincoln et al. 1992). An LOD > 4.0 was used to group markers, and map units were calculated with the Kosambi mapping function (Kosambi 1944).

QTL mapping

All QTL analyses were conducted using composite interval mapping (CIM; Zeng 1994) as executed by the program Zmapqtl (model 6) of the software suite QTL Cartographer version 1.13 (Basten et al. 1994 and 1999). QTL mapping methods such as CIM are as robust and powerful as nonparametric analysis when analyzing categorical traits that exhibit normal or near-normal distributions like ours (see Fig. 1) (Rebai 1997). QTL analysis was conducted separately for each trait in each backcross using means of BC individuals averaged over replicates. Homogeneous error variances allowed data for the traits SP, PI, ST, and DD to be averaged across experiments within detached-leaflet and whole-plant assays for CIM analysis. CIM was run with a 10-cM window and a maximum of 10 marker cofactors per model. Tests were performed at 2-cM intervals, and cofactors were selected by forward–backward stepwise regression using the program SRmapqtl with a critical P value of 0.10. Genome-wide, trait-specific, threshold values (α = 0.05) of the likelihood ratio (LR) test statistic for declaring the presence of a QTL were estimated from 1000 permutations of the means of the BC_1 individuals for each phenotypic trait (Churchill and Doerge 1994; Doerge and Churchill 1996). The phenotypic variation (R^2) explained by a QTL, conditioned by the CIM cofactors included in the model, was calculated at the most likely QTL position. The additive effect of an allelic substitution (H for E) at each QTL was also obtained. LR statistics were converted to LOD scores using the formula LOD = (0.217)LR, according to Basten et al. (1999). One-LOD support intervals for the position of each QTL were calculated from the CIM results. QTL regions across experiments and backcrosses were delineated by overlapping one-LOD support intervals detected in individual experiments (see Fig. 2).

A statistical test to evaluate the correspondence of QTLs was performed using the hypergeometric probability distribution following the procedure of Lin et al. (1995) to calculate the probability of obtaining the observed number of matching QTLs. A QTL match was declared when the one-LOD support intervals overlapped. For the formula in Lin et al. (1995), the number of comparison intervals (n) was 40, based on the average size of our QTLs and the overall map distance (858/22 cM for BC-E and 1082/28 cM for BC-H). This correspondence test was used to compare QTLs detected by different traits in the same assay method, resistance QTLs detected by different assay methods, and resistance QTLs with droplet dispersal QTLs.

Results

Phenotypic trait analysis

Phenotypic distributions for related experiments (i.e., same assay type and trait) were similar and continuous, as expected for quantitatively inherited traits. Representative distributions are shown (Fig. 1). The parents, susceptible NC84173 (E) and resistant LA2099-MD1 (H), were at or near the distribution extremes for all traits, with H exhibiting lower values for disease resistance traits (indicating greater resistance) and greater droplet dispersal than E. The F_1 fell between the parental means and few transgressive segregants were present in either backcross (Fig. 1 and data not shown). In general, the BC-H population was more resistant to *P. infestans* than the BC-E population. Significant skewing ($P \leq 0.05$) was observed in several experiments for the assay–trait combinations: field – stem resistance score (FLD–ST) in BC-E and BC-H; detached-leaflet – droplet dispersal (DL–DD) and detached-leaflet – sporulating lesion length (DL–SP) in BC-E (see Table 1 for abbreviations).

In the ANOVA, the genotype component of variation was always highly significant ($P \leq 0.001$) for all traits (data not shown), indicating BC individuals differed from each other for each trait evaluated. Replication always accounted for the largest proportion of the variation in disease resistance. Broad-sense heritabilities based on BC genotype means for each experiment ranged from 0.29 to 0.81 in BC-E and 0.23 to 0.83 in BC-H. Higher trait heritabilities were observed in detached-leaflet (0.42 to 0.81 in BC-E; 0.41 to 0.80 in BC-H) and whole-plant assays (0.52 to 0.75 in BC-E; 0.50 to 0.83 in BC-H) than for field assays (0.23 to 0.58 in BC-E; 0.26 to 0.39 in BC-H).

Correlations were frequently non-significant when comparing between assay methods (detached-leaflet, whole-plant, or field) (data not shown). When significant, correlations between assay methods were positive, indicating that there may be some genetic factors contributing to resistance in all assay methods. Correlations were generally higher in BC-E than in BC-H. More positive correlations were detected between whole-plant and field assays than between detached-leaflet and field assays.

Fig. 2. Linkage maps showing QTLs detected with CIM for resistance to late blight (*lb*) and inoculum droplet dispersal (*dd*) in backcrosses to *Lycopersicon esculentum* (BC-E, left side) and *Lycopersicon hirsutum* (BC-H, right side). Bars represent one-LOD support intervals and arrows on the bars indicate the most likely QTL position. The peak LOD value and trait-specific significance threshold at a permutated genome-wide significance level of $P \leq 0.05$ are shown below the QTL positions, and are separated by a slash. Commas on the left side of the slash separate LOD values for multiple peaks on the same chromosome for the same trait and experiment. Resistance QTLs detected in individual experiments are labeled by assay type (DL,WP,FLD), experiment (*A* to *K*), and phenotype trait. All notation corresponds to Table 1. QTL regions detected across experiments and backcrosses, as determined by overlapping one-LOD support intervals, are indicated in bold italics between the two backcross chromosomes. These QTL regions are labeled *lb* (disease resistance) or *dd* (droplet dispersal) and by chromosome number, followed by a letter if more than one region could be distinguished. RFLP markers with significant skewing ($P \leq 0.05$) are suffixed with an ampersand (&) when skewed toward the homozygous class and a pound sign (#) when skewed toward the heterozygous class.

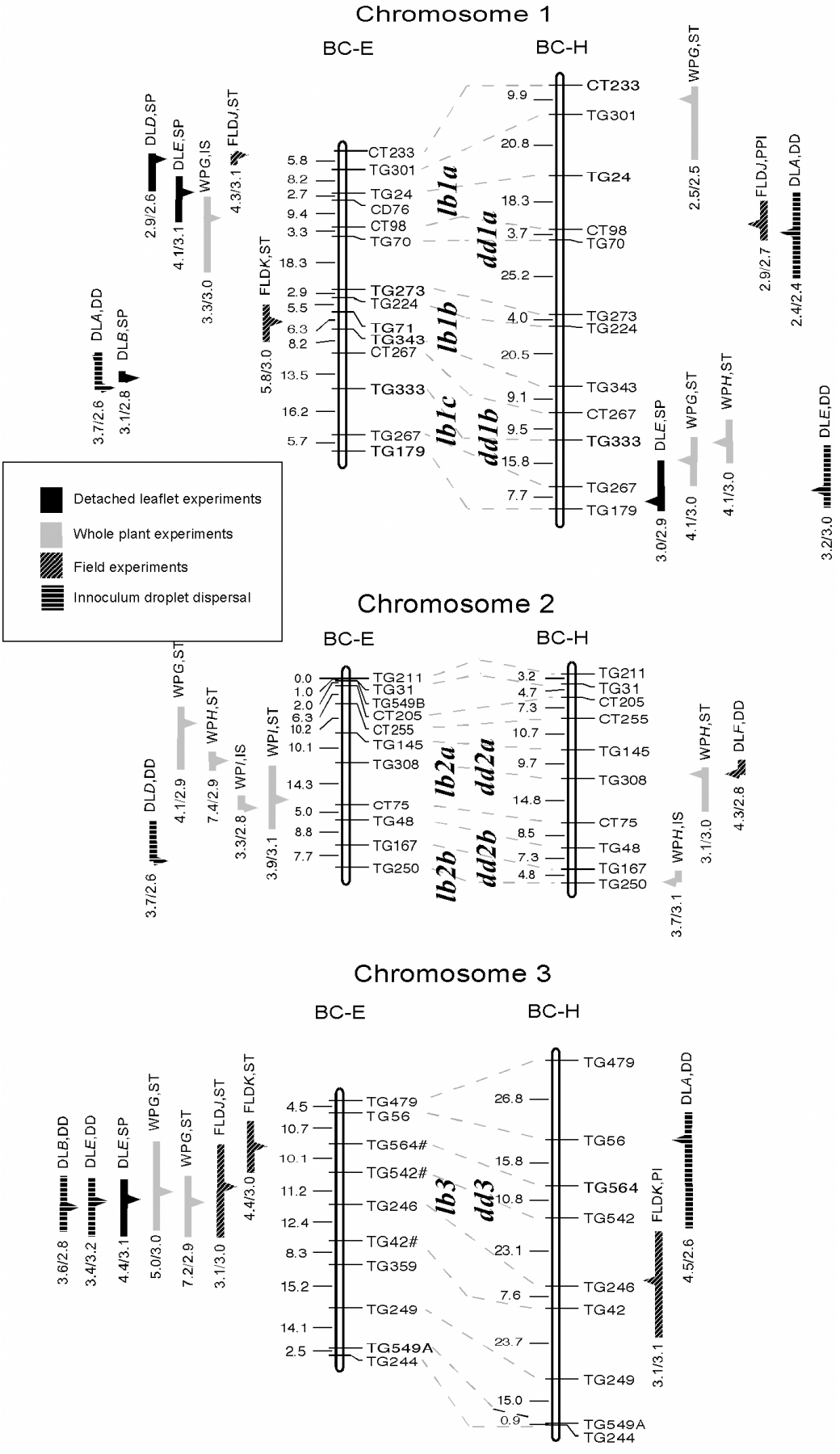


Fig. 2 (*continued*).

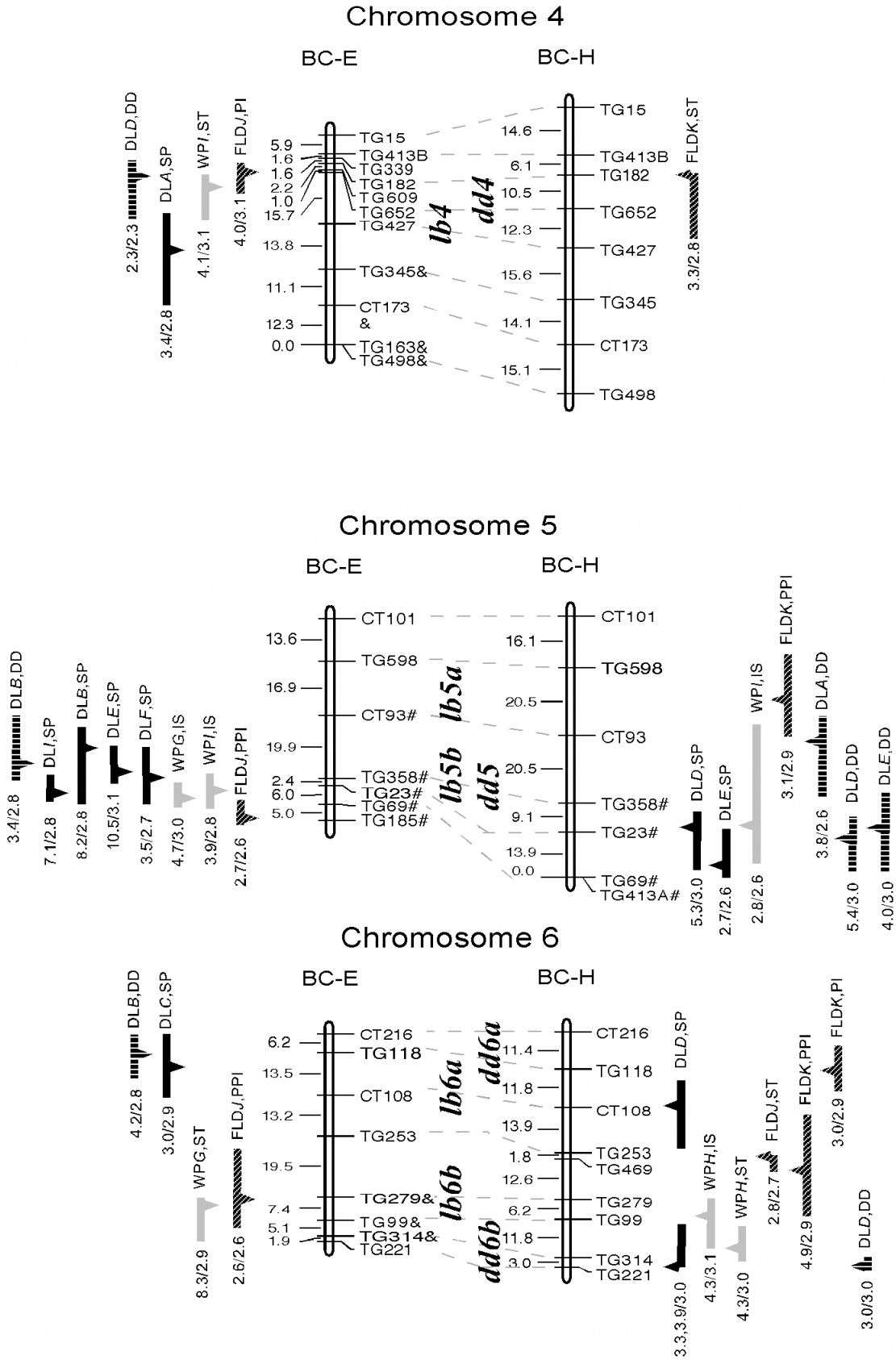


Fig. 2 (*continued*).

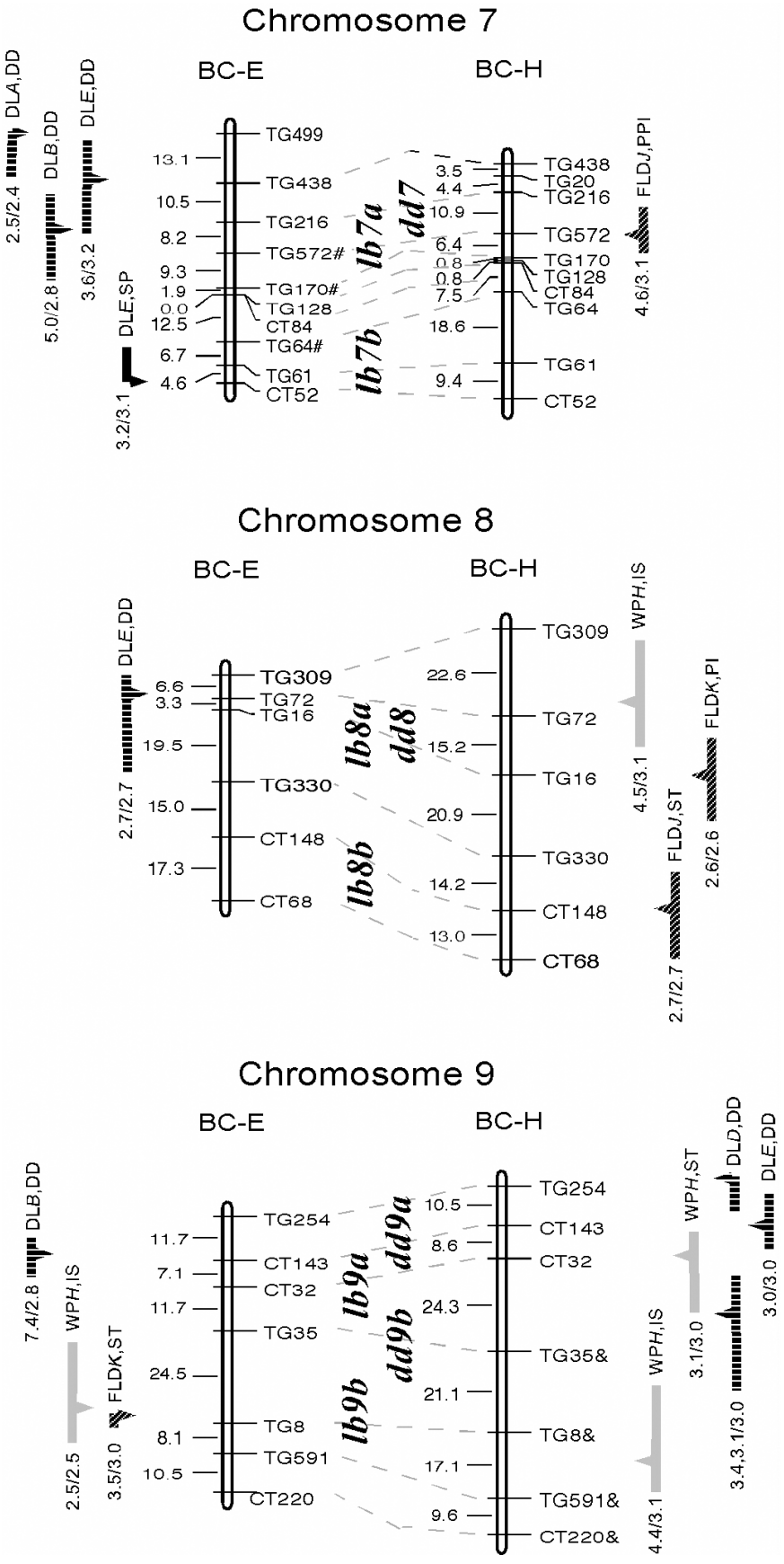
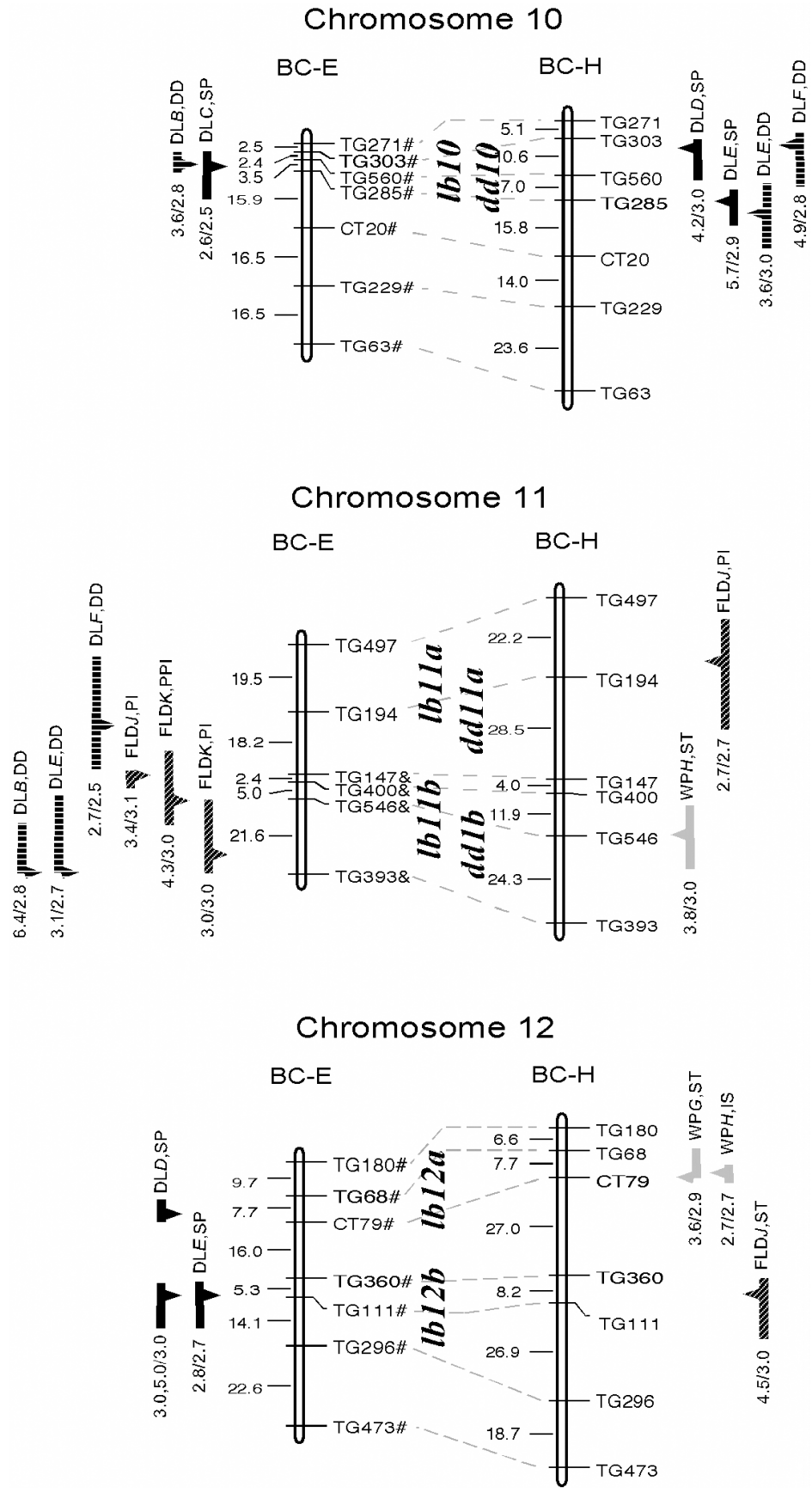


Fig. 2 (*concluded*).



Linkage maps and marker segregation

The BC-E linkage map includes 104 markers on 12 chromosomes (chr) covering 858 cM, with an average spacing of 9.3 cM between markers; and the BC-H map includes 98 markers on 12 chr covering 1082 cM, with an average spacing of 12.6 cM between markers (Fig. 2). No gaps exceeded 25 and 30 cM in the BC-E and BC-H maps, respectively. Marker orders were nearly identical to the high-density *L. esculentum* × *L. pennellii* tomato map (Tanksley et al. 1992), except the order of TG15 and TG413B on chr 4 were reversed in both of our maps. More marker loci deviated significantly ($P \leq 0.05$) from the expected 1:1 segregation ratio in BC-E (32%) than in BC-H (8%) (see Fig. 2). Distorted loci were clustered on chr 3, 4, 5, 7, 10, 11, and 12 in BC-E. Heterozygotes were favored in most skewed intervals in BC-E, and all markers on chrs. 10 and 12 were highly skewed, with some loci having as low as 27% homozygous *L. esculentum* individuals. Clusters of distorted loci were found on chr 5 and 9 in BC-H.

QTLs detected in detached-leaflet (DL) assays

In BC-E, 10 QTLs were mapped for sporulating lesion length (SP) on 8 chr, based on overlapping one-LOD support intervals (Fig. 2; Table 2). *Lb1a*, *lb5b*, and *lb12b* were detected in two or more assays. QTLs *lb1a*, *lb3*, *lb5b*, and *lb12a* explained more than 20% of the phenotypic variation in at least one experiment. When SP was averaged across experiments, *lb1a*, *lb5b*, and *lb7b* were the only significant QTLs identified, and resistance was conferred by the H allele. In BC-H, four QTLs were detected on four chr (Fig. 2; Table 2). QTLs *lb5ab*, *lb6ab*, and *lb10* were each detected in two experiments and explained up to 22% of the phenotypic variation. When SP was averaged across experiments in BC-H, *lb1a*, *lb1c*, *lb5b*, and *lb10* were detected. Resistance at *lb1c*, *lb5b*, and *lb10* was associated with the H allele, but at *lb1a* with the E allele.

QTLs detected in whole-plant (WP) assays

In BC-E, five and four QTLs were detected for infection score (IS) and stem resistance (ST), respectively, and six and seven QTLs were mapped in BC-H for IS and ST, respectively (Fig. 2; Table 2). Only *lb2a* and *lb3* were identified by both IS and ST within each backcross for individual experiments (Table 2), and the probability of chance correspondence between IS and ST QTLs was 0.76 and ~1.00 in BC-E and BC-H, respectively. The most frequently detected QTL in BC-E was *lb2a*, which explained up to 24% of the phenotypic variation and for which resistance was inherited from E. Other QTLs identified in more than one whole-plant experiment were *lb3* and *lb5b* in BC-E, and *lb1c*, *lb6ab*, and *lb12a* in BC-H (Table 2). Using IS and ST averaged across experiments in BC-E, *lb4* was detected for IS, and *lb2a* and *lb3* for ST. In BC-H, *lb6ab*, *lb9b*, and *lb12a* were detected with averaged IS, and *lb1c* was detected with averaged ST. Most QTLs identified frequently in individual assays were also identified using IS and ST averaged across experiments.

QTLs detected in field (FLD) assays

Resistance of plants in field assays was evaluated using percent infection (PPI and PI) and stem resistance (ST). Trait data was not pooled over assays, since the disease pressure was much greater at field site *J*. Eight QTLs were mapped on seven chr in BC-E (Fig. 2; Table 2). QTLs detected more than once across sites or traits were *lb3* and *lb11b*, which described 19% to 27% of the phenotypic variation. *Lb3* was revealed only for ST, and *lb11b* was identified only with PI and PPI. Resistance at both QTLs was contributed by the H allele. In BC-H, 10 QTLs were mapped on nine chr. Only *lb6ab* was detected more than once, accounting for up to 26% of the phenotypic variation (Fig. 2; Table 2). The probability of chance correspondence between QTLs detected for foliage and stem resistance was ~1.00 in both backcrosses.

Resistance QTLs detected across experiments or assays

The resistance QTLs detected across three or more replicated experiments or in all assay methods were *lb1a*, *lb2a*, *lb3*, *lb4*, *lb5b*, and *lb11b* in BC-E and *lb5ab* and *lb6ab* in BC-H (Fig. 2; Table 2). *Lb5ab* was detected by leaf-related traits in all three assay methods in both backcrosses. In detached-leaflet experiments, *lb5b* was detected for multiple experiments and for averaged sporulating lesion length (SP) in both backcrosses. In BC-E, the QTL lies between CT93 and TG185 on chr 5, but in BC-H, it encompassed both *lb5a* and *lb5b* owing to large LOD support intervals (Fig. 2). Resistance was associated with H alleles in both backcrosses, suggesting that *lb5ab* has additive gene action. *Lb1a* was detected by all assay methods in BC-E, by two assay methods in BC-H, and for averaged SP in both backcrosses. Increased resistance was contributed by H alleles in BC-E and E alleles in BC-H. These contrasting parental allele effects may indicate overdominant gene action for the QTL or linked QTLs with dominant effects derived from each parent. *Lb3* and *lb4* were detected in BC-E in all assay methods. Resistance was associated with both stems and leaves, and the H allele. Both QTLs were identified in only one experiment in BC-H, suggesting mainly dominant gene action. *Lb11b* was revealed by field assays in BC-E, with resistance conferred by H alleles (Fig. 2). Since this QTL was significant for multiple experiments in BC-E, but only for one experiment in BC-H, this QTL may have dominant gene action. *Lb11b* was detected only with leaf-related traits in field assays where disease development occurred on mature plants. Resistance was detected at *lb6ab* for all assay methods and for both stems and leaves in BC-H. The only QTL detected in multiple experiments with resistance associated only with the E allele in both BC-E and BC-H was *lb2a*, which was associated with both stem and leaf resistance.

QTLs for developmental traits and coincidence with resistance QTLs

In BC-E, QTLs for determinate growth, total number of nodes, and plant height mapped to chr 6 in the TG279 region (data not shown). The *sp* (self-pruning) locus that controls growth habit is closely linked to TG279 (Grandillo and Tanksley 1996; Pnueli et al. 1998). BC-E segregated for determinate growth habit, but all BC-H individuals were indeterminate. A resistance QTL detected in BC-E in field assay *J* mapped to chr 6, with the peak near TG279 (Fig. 2). Significant negative correlations between percent plot infected (PPI) and plant height were observed ($r = 0.48$, $P \leq 0.001$), suggesting that the *sp* locus affected the disease phenotype.

Table 2. Phenotypic variation (R^2) accounted for and additive effect of allelic substitution at late blight (*lb*) resistance QTLs.

			Detached leaflet assay			Whole plant assay			Field assay		
QTL[a]	Marker interval[b]	Position[c]	No.[d]	R^2 [e]	Effect[e]	No.	R^2	Effect	No.	R^2	Effect
BC-E											
lb1a	CT233-TG273	0–40	2	15, 22	–1.9, –3.1	1	22	–0.51	1	*17*	*–0.90*
lb1b	TG224-TG343	53–67	0			0			1	*33*	*–0.38*
lb1c	TG267-TG333	80–83	1	8	–4.0	0			0		
lb2a	CT255-TG167	10–54	0			4	15 *16, 24, 17*	0.68 *0.29, 0.76, 0.49*	0		
lb3	TG56-TG42	9–49	1	22	–3.2	2	32 *33*	–0.68 *–0.44*	2	*20, 27*	*–0.67, –0.33*
lb4	TG15-CT173	0–52	1	8	2.4	1	*24*	*–0.44*	1	22	–15.5
lb5b	CT93-TG185	31–64	4	14, 34, 22, 17	–3.1, –3.6, –3.2, –5.7	2	19, 14	–0.57, –0.49	1	16	–9.9
lb6a	CT216-CT108	0–19	1	9	3.5	0			0		
lb6b	TG253-TG221	35–67	0			1	*38*	*0.46*	1	15	–8.6
lb7b	TG64-CT52	55–67	1	16	3.3	0			0		
lb9b	TG35-TG591	32–54	0			1	11	–0.44	1	*20*	*0.35*
lb10	TG271-CT20	2–15	1	7	–3.0	0			0		
lb11b	TG194-TG393	27–67	0			0			3	19, 23, 20	–17.6, –6.8, –8.1
lb12a	TG68-CT79	15–19	1	25	–3.5	0			0		
lb12b	TG360-TG296	33–47	2	15, 14	2.8, 3.2	0			0		
BC-H											
lb1a	CT233-TG70	0–52	0			1	*13*	*–0.19*	1	15	5.36
lb1c	CT267-TG179	112–145	1	13	–2.0	2	*18, 19*	*0.25, 0.36*	0		
lb2a	TG145-CT75	31–51	0			1	*14*	*0.29*	0		
lb2b	TG167-TG250	69–74	0			1	17	0.47	0		
lb3	TG542-TG249	57–94	0			0			1	16	–6.7
lb4	TG182-TG427	21–39	0			0			1	*16*	*–0.11*
lb5ab[f]	CT101-TG69	12–80	2	21, 12	–3.7, –2.0	1	14	–0.37	1	19	4.2
lb6ab[f]	CT216-TG221	19–72	2	15, 15	3.1, –3.1	2	19 *19*	0.5*1 0.51*	2	15, 26 *15*	–3.7, 4.6 *0.66*
lb7a	TG216-TG170	15–28	0			0			1	21	–6.7
lb8a	TG309-TG330	3–47	0			1	21	–0.55	1	13	3.5
lb8b	TG330-CT68	63–84	0			0			1	*15*	*0.48*
lb9a	CT143-TG35	14–31	0			1	*14*	–0.29	0		
lb9b	TG35-TG591	55–81	0			1	22	–0.59	0		
lb10	TG303-CT20	5–29	2	17, 22	–3.3, –2.7	0			0		
lb11a	TG497-TG147	6–34	0			0			1	15	–6.8
lb11b	TG400-TG393	59–77	0			1	*17*	*–0.32*	0		
lb12a	CT68-CT79	7–14	0			2	14 *18*	0.53 *–0.25*	0		
lb12b	TG360-TG296	42–57	0			0			1	*23*	*0.82*

Note: For whole-plant and field assays, QTLs detected for percentage infection scores (IS, PPI, and PI) are listed first in normal font, followed by QTLs detected for stem scores (ST) in italics. All significant QTLs are listed by backcross population (BC-E or BC-H) and in experiment order (*A–K*).

[a]QTL regions across experiments and backcrosses were identified by overlapping one-LOD support intervals (see Fig. 2). QTLs were labeled by chromosome and followed by a lower case letter if more than one QTL was located on the same chromosome (as shown on Fig. 2).

[b]Marker intervals are identified by the flanking markers encompassing the overlapping one-LOD support intervals (see Fig. 2).

[c]Position of the QTL interval in cM from the top of the linkage group.

[d]The number of experiment–trait combinations with significant QTL effects within an assay type (DL, WP, FLD).

[e]Phenotypic variation (R^2) and effect were obtained from CIM at the most likely QTL position. The R^2 value was the variation explained by the QTL conditioned on the CIM cofactors included in the model. The effect is the additive effect of substituting an *L. hirsutum* allele for an *L. esculentum* allele; thus, a negative effect indicates that the allele for decreased susceptibility (resistance) was from the *L. hirsutum* parent.

[f]QTL regions on chromosome 5 and 6 could be clearly defined as two QTLs (*a* and *b*) in the BC-E population, but not in the BC-H population. In this table, the QTLs in BC-H are summarized together.

In subsequent disease assessments, a conscious effort was made to exclude the effects of the determinate phenotype by scoring resistance as percent infection in a 30-cm^3 area (PPI) at the inoculation site of each plot.

QTLs for droplet dispersal and coincidence with resistance QTLs

In detached-leaflet assays, droplet dispersal (DD) segregated in the BC-E and BC-H and was significantly ($P \leq 0.05$) negatively correlated with most disease resistance traits, indicating increased dispersal was associated with increased resistance (data not shown). Twelve QTLs were detected for droplet dispersal on 11 chr in BC-E and 9QTLs were detected on 7 chr in BC-H (Fig. 2; Table 3). In both backcrosses, droplet dispersal was associated with the presence of the H allele at all QTLs, except at QTL *dd6a* in BC-E and *dd1a*, *dd3*, and *dd9b* in BC-H. *Dd3*, *dd7*, and *dd11b* were the most repeatedly detected QTLs in BC-E and, individually, explained up to 18% of the phenotypic variation (Table 3). When droplet dispersal was averaged across experiments, *dd7* and *dd9a* were identified, which explained 9% and 11% of the phenotypic variation, respectively. In BC-H, the most frequently detected QTLs were *dd5*, *dd9a*, and *dd10*, which individually accounted for up to 22% of the phenotypic variation (Table 3), and *dd*5 was the only QTL detected by average DD data. Some QTLs mapped by more than one experiment in one backcross population (*dd1b*, *dd3*, *dd5*, *dd9a*, *dd10*) were also revealed by at least one experiment in the other backcross, suggesting additive inheritance (Fig. 2; Table 3).

Dispersal QTLs frequently mapped to, or overlapped with, the regions containing QTLs for late blight resistance (Fig. 2). In BC-E, *dd3* and *dd11b* coincided with *lb3* and *lb11b*, two of the most frequently detected resistance QTLs (Fig. 2). Most dispersal QTLs in both backcrosses coincided or overlapped with resistance QTLs (Fig. 2). In BC-H, *dd5* and *dd10* mapped to *lb* QTLs detected in multiple experiments. The probability of chance correspondence between *dd* QTLs and *lb* QTLs was 0.0066 and 1×10^{-6} in BC-E and BC-H, respectively. The probability of chance correspondence of *lb* QTLs with *dd* QTLs remained small (<0.01) when resistance was separated by assay method. Increased inoculum droplet dispersal was consistently associated with an increase in late blight resistance.

Discussion

QTLs for quantitative resistance to *P. infestans* in tomato

To our knowledge, our study is the first to report the detection and localization of QTLs for quantitative resistance to *P. infestans* in tomato. A total of 15 and 18 QTLs were mapped in the BC-E and BC-H populations, respectively (Table 2). Each QTL may contain multiple genetic factors that were not detected individually (Doerge 2002). Our QTL mapping analysis confirmed that genetic control of resistance to *P. infestans* contributed by *L. hirsutum* LA2099-MD1 is quantitatively inherited, multigenic, and environmentally influenced. The most consistently detected resistance QTLs in replicated experiments or across assay methods were mapped within QTL regions *lb1a*, *lb2a*, *lb3*, *lb4*, *lb5b*, and *lb11b* in BC-E and *lb5ab* and *lb6ab* in BC-H (Fig. 2; Table 2). These QTLs were also among the strongest QTLs detected in individual experiments across all assay methods, based on R^2 values and LOD scores (Fig. 2; Table 2). The remaining QTLs were not consistently detected across assay methods, suggesting the environment influences expression of disease resistance at these loci. Environmental variation can affect both QTL detection and localization, especially when it is large relative to genetic variation (i.e., low heritability) (Visscher et al. 1996; Mackay 2001). Owing to environmental variability and low-to-moderate correlations among assay methods (data not shown), replicated experiments and multiple disease assays were essential for mapping quantitative resistance QTLs (Fig. 2; Table 2).

The expression of resistance may also vary among plant organs and by plant age. The probability that QTLs detected for stem and leaf resistance corresponded by chance alone was high for whole-plant and field assays in both backcross populations, suggesting that different genes may control resistance in different organs, at least partly. Among the more consistently detected QTLs, *lb5b* and *lb11b* in BC-E were only associated with leaf resistance, but *lb1a*, *lb2a*, *lb3*, and *lb4* in BC-E and *lb5ab* and *lb6ab* in BC-H were all associated with both leaf and stem resistance. Similarly, resistance to *P. capsici* in pepper was conferred both by QTLs contributing to only one component of resistance and QTLs affecting multiple resistance components (Lefebvre and Palloix 1996). The degree of resistance to *P. infestans* can change as plants mature. Potato cultivars tend to be more susceptible when young and at the initiation of flowering (Grainger 1956; Thurston 1971). Resistance contributed by the *Ph2* locus increases as tomato plants age (Turkensteen 1973; Moreau et al. 1998). Plant maturity can affect the detection of *P. infestans* resistance QTLs, as documented in potato (Collins et al. 1999). The expression of *lb11b* may be maturity-dependent, since this QTL was only detected for percent infection (PI and PPI) in field assays with mature plants.

Resistance QTLs detected across disease phenotype assays

Disease resistance assays conducted in growth chambers and greenhouses can permit rapid, environmentally controlled evaluation of many plant genotypes and pathogen isolates. However, the value of these assays directly depends on their ability to accurately reflect plant resistance to *P. infestans* under field conditions. In potato, both detached-leaflet and whole-plant assays are routinely used to evaluate resistance to *P. infestans*, particularly that based on isolate-specific R genes (Leonards-Schippers et al. 1994; Kuhl et al. 2001; Naess et al. 2001). In our study, the probability of chance correspondence between resistance QTLs detected by different assay methods was less than 0.05 only when comparing QTLs for whole plant and field in BC-E and detached leaflet and whole plant in BC-H. However, many QTL intervals were detected in all assay methods in BC-E (Table 2). Thus, our results indicate that neither detached-leaflet nor whole-plant assays can entirely substitute for field assays when evaluating quantitative resistance to *P. infestans* in tomato. Recent studies of quantitative resistance in potato have also relied on field assays (Colon et al. 1995; Collins et al. 1999; Ewing et al. 2000; Ghislain et al. 2001).

Table 3. Phenotypic variation (R^2) accounted for and additive effect of allelic substitution at inoculum droplet dispersal (*dd*) QTLs.

			Droplet dispersal		
QTL[a]	Marker interval[b]	Position[c]	No.[d]	R^{2e}	Effect[e]
BC-E					
dd1b	CT267-TG333	76–82	1	9	0.26
dd2b	TG48-TG250	50–65	1	19	0.28
dd3	TG542-TG42	29–47	2	9, 18	0.24, 0.31
dd4	TG182-TG427	10–25	1	12	0.23
dd5	CT93-TG358	28–43	1	8	0.24
dd6a	CT216-CT108	0–14	1	10	–0.26
dd7a	TG499-TG170	0–35	3	5, 13, 18	0.14, 0.33, 0.31
dd8a	TG309-TG330	0–23	1	14	0.24
dd9a	TG254-CT32	7–15	1	18	0.47
dd10	TG271-TG285	2–8	1	9	0.24
dd11a	TG497-TG147	5–34	1	16	1.44
dd11b	TG546-TG393	44–67	2	15, 16	0.34, 0.28
BC-H					
dd1a	TG24-TG273	37–67	1	9	–0.28
dd1b	TG333-TG179	123–145	1	14	0.27
dd2a	TG145-TG308	31–36	1	19	0.42
dd3	TG479-TG542	20–49	1	18	–0.60
dd5	TG598-TG69	26–78	3	13, 22, 16	0.34, 0.38, 0.30
dd6b	TG314-TG221	69–73	1	13	0.38
dd9a	TG254-CT32	0–19	2	13, 13	0.30, 0.30
dd9b	CT32-TG8	25–51	1	19	–0.41
dd10	TG271-CT20	4–35	2	18, 21	0.31, 0.32

[a]QTL regions across experiments and backcrosses were identified by overlapping one-LOD support intervals (see Fig. 2). QTLs were labeled by chromosome and followed by a lower case letter if more than one QTL was located on the same chromosome (as shown on Fig. 2).

[b]Marker intervals are identified by the flanking markers encompassing the overlapping one-LOD support intervals (see Fig. 2).

[c]Position of the QTL interval in cM from the top of the linkage group.

[d]The number of experiment/trait combinations with significant QTL effects within an assay type (DL, WP, FLD).

[e]Phenotypic variation (R^2) and effect were calculated at the most likely QTL position. The R^2 was calculated as the variation explained by the QTL conditioned on the CIM cofactors included in the model. Effect is the additive effect of substituting an *L. hirsutum* allele for an *L. esculentum* allele; thus, a positive effect indicates increased droplet dispersal was conferred by the H allele.

Comparing QTLs for *P. infestans* resistance in tomato with potato

QTLs detected in tomato can be compared with those in potato, since both are hosts to *P. infestans*, and the closely related *Solanum* and *Lycopersicon* genomes are highly syntenic, differing by five inversions (Tanksley et al. 1992; Grube et al. 2000). In potato, QTLs for quantitative foliar resistance to *P. infestans* have been identified on every potato chromosome (Leonards-Schippers et al. 1994; Collins et al. 1999; Oberhagemann et al. 1999; Ewing et al. 2000; Sandbrink et al. 2000; Ghislain et al. 2001; Gebhardt and Valkonen 2001). These potato studies used replicated disease phenotype assays to reveal the contribution of multiple QTLs to late blight resistance. Overall, our QTL results were comparable with those found in potato in terms of the number and genomic distribution of resistance QTLs detected and the amount of phenotypic variation explained by these QTLs (Leonards-Schippers et al. 1994; Collins et al. 1999; Oberhagemann et al. 1999; Ghislain et al. 2001). Three regions of the potato genome (middle of chr III, top of chr IV, and top of chr V) were most consistently associated with foliage resistance in multiple mapping experiments using genetically diverse potato populations, and these QTLs often explained a large amount of the phenotypic variation (Collins et al. 1999; Gebhardt and Valkonen 2001).

Linkage maps with molecular markers in common can be used for comparing locations of mapped resistance QTLs across the Solanaceae (Grube et al. 2000). We focused our comparisons on the most consistently detected *P. infestans* resistance QTLs in tomato and potato. Based on common RFLP markers, *lb3* corresponds to the genomic region on potato chr III identified with *P. infestans* resistance. An inversion between tomato chr 5 and potato chr V complicates the comparison, but *lb5b* does not appear to be coincident with the potato late blight resistance QTL on chr V. *Lb4* was coincident with the potato *P. infestans* resistance QTL on chr IV, and *lb4* was detected in field and whole-plant assays in both backcrosses. Several potato late blight resistance QTLs

coincided with the *lb11b* interval, but none of these potato QTLs explained a large portion of the phenotypic variation (Collins et al. 1999; Ghislain et al. 2001). The potato studies identified few resistance QTLs on potato chromosome intervals corresponding to *lb1a* and *lb2a*. Resistance at *lb1a* may be related to specific developmental traits, such as the self-incompatibility (*S*) locus located near CD76 on tomato chr 1 (Bernacchi and Tanksley 1997). The colocalization of quantitative resistance QTLs on chr III and IV in potato and tomato can imply conservation of resistance loci after evolutionary divergence of the two genera. The same functional gene or gene families or, alternatively, functionally different, but closely linked, genes may be responsible for the observed QTL effects (Grube et al. 2000).

Comparing QTLs for *P. infestans* resistance with R genes

Chromosomal regions associated with QTLs for quantitative resistance can coincide or overlap with R genes controlling qualitative resistance (Grube et al. 2000; Caranta et al. 1997; Li et al. 1999; Oberhagemann et al. 1999; Tabien et al. 2002). A comparative mapping study of disease resistance genes across the Solanaceae (pepper, potato, and tomato) revealed that R genes tended to cluster together (Grube et al. 2000). Disease and pest-resistance gene clusters appear to be in chromosomal positions coincident with our resistance QTLs *lb1a*, *lb3*, *lb4*, *lb5b*, and *lb11b*. *Lb4* and *lb11b* also seemed to co-localize with clusters in potato that contain R loci known to confer isolate-specific resistance to *P. infestans* (Grube et al. 2000; Gebhardt and Valkonen 2001). This colocalization supports the hypothesis that at least some resistance QTLs may be alleles at R loci or have evolved from clusters of duplicated R genes. "Defeated" R genes have been shown to act as QTLs conferring partial resistance to *Xanthomonas oryzae* in rice (Li et al. 1999). Although Grube et al. (2000) found that resistance gene clusters were generally conserved across genera in the Solanaceae, the only two regions of conserved function (top of chr 4 coincident with *lb4* and bottom of chr 11 coincident with *lb11b*) were for resistance to *Phytophthora* species in both potato and pepper. Our results now extend the possible functional conservation of some resistance loci in the Solanaceae to QTLs for resistance to *P. infestans* in tomato.

Comparisons were made to determine if our resistance QTLs colocalized with three previously mapped R genes in tomato for isolate-specific resistance to *P. infestans* (Pierce 1971; Moreau et al. 1998; Chunwongse et al. 1998). QTLs were detected at or near the reported locations of two *Ph* loci (*Ph1* on chr 7 with *lb7a* in BC-H; *Ph3* on chr 9 with *lb9b* in both backcrosses). However, these resistance QTLs were detected by only one (*lb7a*) or two (*lb9b*) experiments, providing limited evidence for possible R gene involvement in the expression of some resistance QTLs. Interestingly, none of our most consistently detected QTLs (*lb1a*, *lb2a*, *lb3*, *lb5b*, and *lb11b* in BC-E and *lb5ab* and *lb6ab* in BC-H) appeared associated with any of the *Ph* loci, suggesting that these QTLs represent a novel source of *P. infestans* resistance in tomato.

QTLs for plant developmental traits and relation to resistance

QTLs for determinate growth, number of nodes, and plant height in BC-E all mapped to chr 6 in the region of *lb6b* (Fig. 2 and data not shown). This region also includes the *sp* (self-pruning) locus, which controls determinate (compact) growth habit (Pnueli at al. 1998; Weide et al. 1993). Plant height was significantly negatively correlated with percent infection, and determinate (*sp*/*sp*) plants were more susceptible than indeterminate (*Sp*/-) plants. Thus, *lb6b* may result from pleiotropic effects of the *sp* locus. Associations between developmental traits and *P. infestans* resistance may also be due to linkage or pleiotropic effects of developmental QTLs on resistance.

QTLs for droplet dispersal and relation to resistance

A strong association between increased droplet dispersal and greater late blight resistance in detached-leaflet assays was revealed by trait correlations and the coincidence of many droplet dispersal (*dd* QTLs) and *lb* QTLs in BC-E and BC-H (Fig. 2; Tables 2 and 3). The relationship of dispersal with resistance in whole-plant and field assays was somewhat weaker: some *dd* QTLs mapped to intervals where no *lb* QTLs were detected in whole-plant and field assays. Significant correspondence was also detected between *lb* and *dd* QTLs in both backcrosses and all assay methods, suggesting that direct selection for increased droplet dispersal on leaves may increase resistance.

Rapid droplet dispersal may reduce leaflet surface moisture, possibly preventing or impeding *P. infestans* growth, or dispersal may reduce *P. infestans* exposure to leaf compounds that promote infection. Free water is required for *P. infestans* sporulation and zoospore germination, and moist, cool conditions are required for optimal disease development (Bold et al. 1987). Leaves of *L. hirsutum* differ from *L. esculentum* by secondary metabolites and by trichome type and density, traits implicated in insect resistance (Farrar and Kennedy 1991). Trichome type and density may influence surface tension directly, or by differences in exudates.

Late blight resistance QTLs and marker-assisted selection (MAS)

The mapping of quantitative resistance QTLs provides the basis for using MAS to introgress resistance alleles from wild species into cultivated tomato to improve late blight resistance. The simultaneous use of reciprocal backcross populations for QTL mapping in our study also permitted the detection of QTL alleles associated with dominant effects on the resistance phenotype. Recent empirical studies have confirmed that MAS for favorable alleles at QTLs controlling traits with low-to-moderate heritability can result in appreciable selection gains (Ahmadi et al. 2001; Robert et al. 2001; Yousef and Juvik 2002). Both simulated and empirical results indicate that MAS can be superior to phenotypic selection for low heritability traits, particularly when traits are difficult or costly to phenotype (Hospital and Charcossett 1997; Yousef and Juvik 2002). The environmental variability detected between experiments and assay methods in our study indicates that costly replications are necessary to accurately assess resistance to late blight, suggesting that MAS would be of benefit for genetic improvement efforts.

The most promising resistance QTLs identified here for improving resistance to *P. infestans* in cultivated tomato are *lb1a*, *lb3*, *lb4*, *lb5b*, and *lb11b*, detected in BC-E, with resistance

conferred by the H allele. Introgression of the *L. hirsutum* allele at one or more of these QTLs may provide a beneficial level of reduction in disease development, as was observed when *Lycopersicon cheesmanii* alleles for resistance to *Alternaria alternata* at selected QTLs were introgressed into cultivated tomato (Robert et al. 2001). Even modest improvements in disease resistance can delay or eliminate the need for fungicide applications. Models predicting late blight disease development in potato include cultivar resistance level as an important factor (Fry et al. 1983). Using MAS to pyramid quantitative resistance QTLs and qualitative R genes in carefully selected combinations within the same cultivar may enhance the durability of resistance by limiting the growth of any *P. infestans* isolates that overcome the R genes (Tabien et al. 2002; Young 1996).

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