Theor Appl Genet (2004) 108:628–638
DOI 10.1007/s00122-003-1469-8

ORIGINAL PAPER

D. J. Brouwer · D. A. St. Clair

Fine mapping of three quantitative trait loci for late blight resistance in tomato using near isogenic lines (NILs) and sub-NILs

Received: 11 June 2003 / Accepted: 1 September 2003 / Published online: 28 October 2003

Abstract An earlier study identified quantitative trait loci (QTLs) *lb4*, *lb5b*, and *lb11b* for quantitative resistance to *Phytophthora infestans* (late blight) in a backcross population derived from crossing susceptible cultivated tomato (*Lycopersicon esculentum*) with resistant *L. hirsutum.* The QTLs were located in intervals spanning 28–47 cM. Subsequently, near-isogenic lines (NILs) were developed for *lb4*, *lb5b*, and *lb11b* by marker-assisted backcrossing to *L. esculentum*. Sub-NILs containing overlapping *L. hirsutum* segments across each QTL region were selected and used to validate the QTL effects, fine-map QTLs, and evaluate potential linkage drag between resistance QTLs and QTLs for horticultural traits. The NILs and sub-NILs were evaluated for disease resistance and eight horticultural traits at three field locations. Resistance QTLs were detected in all three sets of NIL lines, confirming the BC_1 mapping results. *Lb4* mapped near TG609, and between TG182 and CT194, on chromosome 4, a 6.9-cM interval; *lb5b* mapped to an 8.8-cM interval between TG69a and TG413 on chromosome 5, with the most likely position near TG23; and *lb11b* mapped to a 15.1-cM interval on chromosome 11 between TG194 and TG400, with the peak centered between CT182 and TG147. Most QTLs for horticultural traits were identified in intervals adjacent to those containing the late blight resistance QTLs. Fine mapping of these QTLs permits the use of marker-assisted selection for the precise introgression of *L. hirsutum* segments containing late blight resistance alleles separately from those containing deleterious alleles at horticulturally important QTLs.

Communicated by D.B. Neale

D. J. Brouwer · D. A. St. Clair (✉)
Department of Vegetable Crops,
University of California,
Davis, CA 95616, USA
e-mail: dastclair@ucdavis.edu
Tel.: +1-530-7521740
Fax: +1-530-7529659

Electronic Supplementary Material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s00122-003-1469-8

Introduction

Late blight, caused by the oomycete *Phytophthora* infestans (Mont.) de Bary, can be a devastating disease to both cultivated tomato (*Lycopersicon esculentum*) and potato (*Solanum tuberosum*), frequently leading to severe crop losses (Fry and Goodwin 1997). New clonal lineages of *P. infestans* have been detected in the United States since the 1990s. The new isolates are resistant to the fungicide metalaxyl, include both the A1 and A2 mating types, which may permit sexual recombination, and can be highly aggressive on tomato (Fry and Goodwin 1997). Varieties with genetic resistance to late blight are desired to control the economic and environmental costs of fungicide applications and reduce crop losses.

Genetic resistance to *P. infestans* can be divided into two broad categories: qualitative and quantitative. Qualitative resistance is typified by a hypersensitive response (HR) that is usually conferred by a single dominant R gene in an isolate-specific, gene-for-gene interaction with pathogen virulence factors. Eleven qualitative resistance genes (*R1* to *R11*) were identified in the wild potato species *Solanum demissum* (Gebhardt and Valkonen 2001), and *Ph1* and *Ph2* were identified in wild tomato species (Gallegly 1960; Moreau et al. 1998). Unfortunately, when these R genes were deployed in cultivars, new *P. infestans* isolates capable of overcoming the resistance conferred by R genes quickly evolved, limiting their usefulness (Wastie 1991). Although additional qualitative resistance genes have been recently identified in tomato (Chunwongse et al. 1998) and potato (Naess et al. 2000; Kuhl et al. 2001), the effectiveness and durability of these genes for control of *P. infestans* remains to be established.

Quantitative resistance (i.e., partial resistance) tends to be multigenic, quantitatively inherited, and can be isolate non-specific (Wastie 1991). No HR is observed, and the net effect is to reduce inoculum and slow the spread of the pathogen. Studies of the genetic basis of quantitative resistance have benefited from the development of molecular marker mapping techniques. Using interspecific populations developed with resistant wild diploid *Solanum* species and quantitative trait locus (QTL) mapping, researchers have identified genetic factors contributing to quantitative resistance to *P. infestans* on all potato chromosomes (e.g., Collins et al. 1999; Oberhagemann et al. 1999; Ewing et al. 2000). All of these studies identified a major quantitative resistance QTL on potato chromosome V. However, this same interval was also associated with undesirable QTLs for late maturity and low plant vigor (Collins et al. 1999; Oberhagemann et al. 1999; Visker et al. 2003). Co-localization of maturity and resistance QTLs may be due to pleiotropic effects of a single gene or linkage between the resistance QTLs and undesirable maturity gene(s). If resistance QTLs are tightly linked to genes for undesirable traits, linkage drag can greatly complicate the use of wild species QTL alleles in breeding efforts.

We identified QTLs for quantitative resistance to late blight in a tomato backcross (BC_1) mapping population (BC-E) developed by crossing susceptible cultivated *Lycopersicon esculentum* line NC 84173 with a single resistant plant (MD1) from wild *Lycopersicon hirsutum* accession LA2099 (Brouwer et al., in press). Between 73 and 213 BC-E plants were phenotyped in three types of replicated disease assays (six detached-leaflet, three whole-plant, and two field assays). Detached leaflet assays measured foliar resistance, while both stem and foliar resistance were evaluated in the whole-plant and field assays. Quantitative resistance QTLs were identified on 11 of the 12 tomato chromosomes in BC-E using composite interval mapping. No QTLs were identified across all replicates; however, six QTLs (*lb1a*, *lb2a*, *lb3*, *lb4*, *lb5b*, and *lb11b* on chromosomes 1, 2, 3, 4, 5, and 11, respectively) were the most consistently identified QTLs across assay methods (Brouwer et al., in press). *Lb1a* co-localized with the *S* locus for self-incompatibility, the cultivated parent contributed resistance at *lb2a*, and resistance was associated with the *L. hirsutum* alleles at *lb3*, *lb4*, *lb5b*, and *lb11b*. QTLs *lb3*, *lb4*, *lb5b*, and *lb11b* for quantitative resistance to late blight co-localized with resistance gene clusters containing genes for resistance to *P. infestans* and other pathogens (Grube et al. 2000).

Refining the estimated position and effect of resistance QTLs enhances their potential use in breeding by marker-assisted selection (MAS) and facilitates map-based cloning, but it requires more detailed analysis than provided by BC_1 mapping. Estimation of QTL effects are biased in mapping populations of limited sample size, and QTL positions are imprecisely estimated (Utz et al. 2000). Multiple genes segregating in mapping populations can also complicate genetic estimates (Yamamoto et al. 1998). QTL effects can also change during introgression of favorable wild alleles into a new cultivated genetic background or due to loss of favorable epistatic interactions between the QTLs and other genes from the wild germplasm segregating in the mapping population. Furthermore, the QTL intervals may actually contain multiple linked genes which all contribute to the observed resistance phenotype.

Near-isogenic lines (NILs) and sub-NILs containing part of the introgressed region present in the NIL have been used in plants to validate and fine map QTLs, since all phenotypic variation can be associated with the lone introgressed allele (Paterson et al. 1990; Alpert and Tanksley 1996; Bernacchi et al. 1998a). NILs and sub-NILs have also been employed to identify multiple genetic factors contributing to a single QTL (Graham et al. 1997; Saito et al. 2001), indicate pleiotropic effects and the physiological basis of QTL effects (Saito et al. 2001), and separate genes for desirable traits from linked genes for undesired traits (Monforte and Tanksley 2000). The BC_1 chromosome intervals containing *lb3*, *lb4*, *lb5b*, and *lb11b* are large (28–47 cM) and are likely to contain numerous genes, some of which may have undesirable alleles from wild *L. hirsutum*, which exhibits many poor horticultural traits. We developed NILs and sub-NILs for four QTLs conferring resistance to late blight in tomato (*lb3*, *lb4*, *lb5b*, and *lb11b*). In this paper, we report the validation and fine mapping of QTLs *lb4*, *lb5b,* and *lb11b* and identify genes for major horticultural traits in the introgressed intervals. The development of a full set of overlapping recombinant sub-NILs for *lb3* was precluded by severe segregation distortion and complete infertility of some homozygous sub-NILs. Consequently, additional experiments to fine map loci controlling segregation distortion and sterility associated with the *lb3* interval were performed and the late blight resistance fine mapping results for *lb3* are reported in a separate manuscript.

Materials and methods

Plant materials

A single BC_1 plant ($BC\text{-}E_{156}$), intact for *L. hirsutum* alleles at four late blight resistance QTLs (*lb3*, *lb4*, *lb5b*, and *lb11b*) on chromosomes 3, 4, 5, and 11, respectively, was selected from our BC_1 mapping population, BC-E (Brouwer et al., in press). BC-E was developed by backcrossing a single F_1, derived by crossing susceptible cultivated *L. esculentum* line NC 84173 (Gardner 1992) and MD1, a single resistant plant (MD1) from self-incompatible, green-fruited *L. hirsutum* accession LA2099, to NC 84173 (Brouwer et al., in press). NILs for each QTL were developed using three backcrosses to *L. esculentum* cv. Hypeel 45, a susceptible tomato cultivar with good horticultural characteristics (Seminis Vegetable Seeds, Woodland, Calif.). During each backcross generation, foreground MAS maintained the heterozygous *L. hirsutum* allele in the target genomic region for each QTL, and background MAS permitted the recovery of homozygous *L. esculentum* genotypes outside of the QTL interval. Foreground MAS was performed using three PCR-based markers per QTL interval, and background MAS was performed using 32 additional markers distributed across the genome (see "Molecular genotyping"). One BC_2 plant heterozygous at DNA markers spanning all

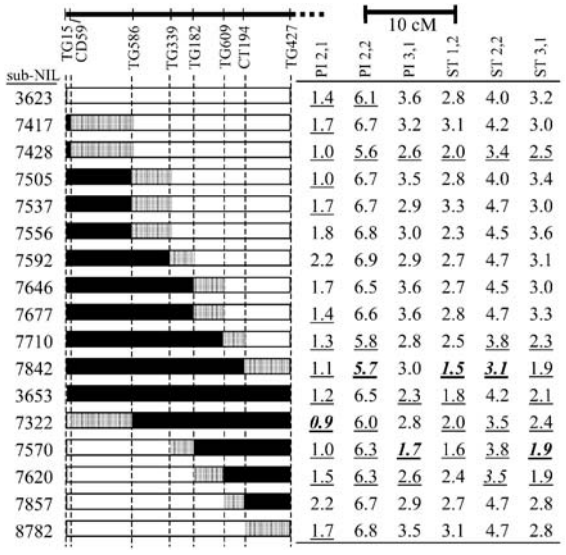

Fig. 1 Graphical genotypes and late blight resistance phenotype (percent infection, *PI* and stem score, *ST*) means for sub-NILs covering the *Lycopersicon hirsutum* introgression on NIL4. *Open bars* represent homozygous *L. esculentum*, *solid bars* represent homozygous *L. hirsutum*, and *gray bars* represent intervals containing crossovers. PI and ST were scored at three locations (1, 2, 3) on two dates (1, 2). Only LSMEANS from ANOVAs with significant genotype effects are shown for each sub-NIL. The most resistant sub-NIL in each column is shown in *bold*, and lines significantly different from the average of the susceptible controls (NC 84173 and Hypeel 45) are *underlined*

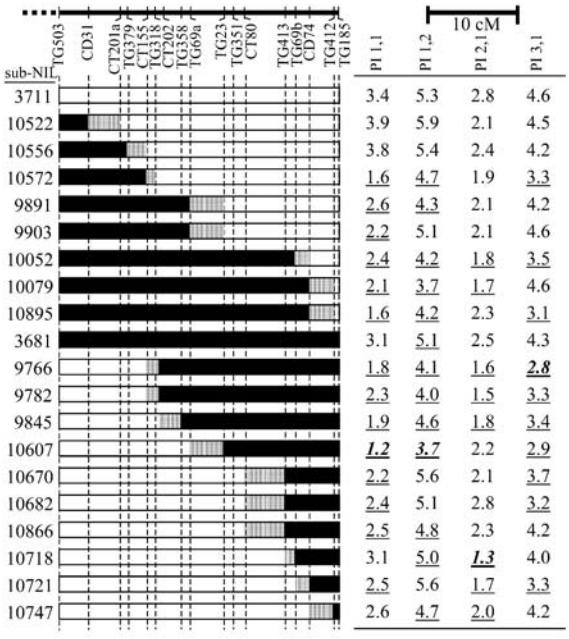

Fig. 2 Graphical genotypes and late blight resistance phenotype (*PI* and *ST*) means for sub-NILs covering the *L. hirsutum* introgression on NIL5. *Open bars* represent homozygous *L. esculentum*, *solid bars* represent homozygous *L. hirsutum*, and *gray bars* represent intervals containing crossovers. PI and ST were scored at three locations (1, 2, 3) on two dates (1, 2). Only LSMEANS from ANOVAs with significant genotype effects are shown for each sub-NIL. The most resistant sub-NIL in each column is shown in *bold*, and lines significantly different from the average of the susceptible controls (NC 84173 and Hypeel 45) are *underlined*

four QTLs was selected from 930 plants and backcrossed to Hypeel 45. Three plants, each heterozygous for one of the *L. hirsutum* QTL intervals, were selected from among 1,000 BC_3 plants, and 150 BC_4 plants were screened to identify NILs for each QTL. The marker-selected NILs contained a single heterozygous *L. hirsutum* segment spanning the *lb4*, *lb5b,* or *lb11b* QTL intervals in an otherwise *L. esculentum* genetic background and were designated NIL4, NIL5, and NIL11, respectively.

Subsequently, NIL4, NIL5, and NIL11 were individually backcrossed to Hypeel 45 to derive three populations of sub-NILs for fine mapping QTLs for resistance and horticultural traits. Markers spanning each introgressed interval were genotyped in 504, 519, and 428 BC_5 plants for NIL4, NIL5, and NIL11, respectively. For each QTL, a set of sub-NILs, plants containing overlapping *L. hirsutum* segments spanning a QTL region, were selected in the BC_5 and allowed to self-pollinate in the greenhouse. NIL and sub-NILs homozygous for *L. hirsutum* segments were then marker-selected by screening 20 to 40 BC_5S_1 plants. The homozygous NILs and sub-NILs were allowed to self-pollinate in the greenhouse in 2001 to provide BC_5S_2 seed for replicated field experiments in 2002.

Molecular genotyping

Genotypes were determined using restriction fragment length polymorphisms (RFLPs) and PCR-based cleaved amplified polymorphic (CAP, Konieczny and Ausubel 1993) markers. For each QTL interval, one central and two flanking RFLP markers were converted to CAP markers as described by Robert et al. (2001). CAP markers were developed for TG15, TG609, and TG427 on NIL4; TG503, TG358, and TG185 on NIL5; and TG194, TG400, and TG393 on NIL11 (Figs. 1, 2, 3). Markers for background selection were chosen based on the graphical genotype of donor plant BC-E_{156}. At least two markers were used to track each heterozygous interval in BC-E_{156}, and markers were separated by less than 25 cM. Additional markers for fine mapping were selected from the high-density tomato map (Pillen et al. 1996). The linkage map position and sequences of RFLP probes are available at http://www.sgn.cornell.edu. A total of 8, 18, and 14 markers were fine-mapped in NIL4, NIL5, and NIL11, respectively.

Genomic DNA was extracted from young leaves using a CTAB mini-prep procedure (Fulton et al. 1995). For RFLP markers, restriction enzyme digestion and Southern analysis was performed as described previously (Truco et al. 2000). Primer sequences and optimized PCR conditions for CAP markers are available online (see Electronic Supplementary Material). Linkage maps were constructed for each NIL separately using MAPMAKER/EXP 3.0 (Lincoln et al. 1992) using the Kosambi mapping function.

Trait phenotyping

Three sets of homozygous NIL and sub-NILs (subsequently referred to as NIL4, NIL5, and NIL11) were evaluated at three northern California field locations near Salinas (location 1), Majors (location 2), and Swanton (location 3). All three coastal locations typically have cool and humid conditions conducive to late blight development. The Salinas field location was level land on Chular loam, the Majors site was sloped on Watsonville sandy loam, and the Swanton site was on Soquel silty clay loam in a narrow valley. The field trial was organized as a split-plot design with NIL sets as whole plots and sub-NILs within a NIL set as sub-plots. Each NIL was randomly assigned to the whole plots and the respective sub-NILs were randomized within a whole plot. Four replicates were planted at each location. Each whole plot also contained late blight resistant MD1 and susceptible NC 84173 and Hypeel 45 as

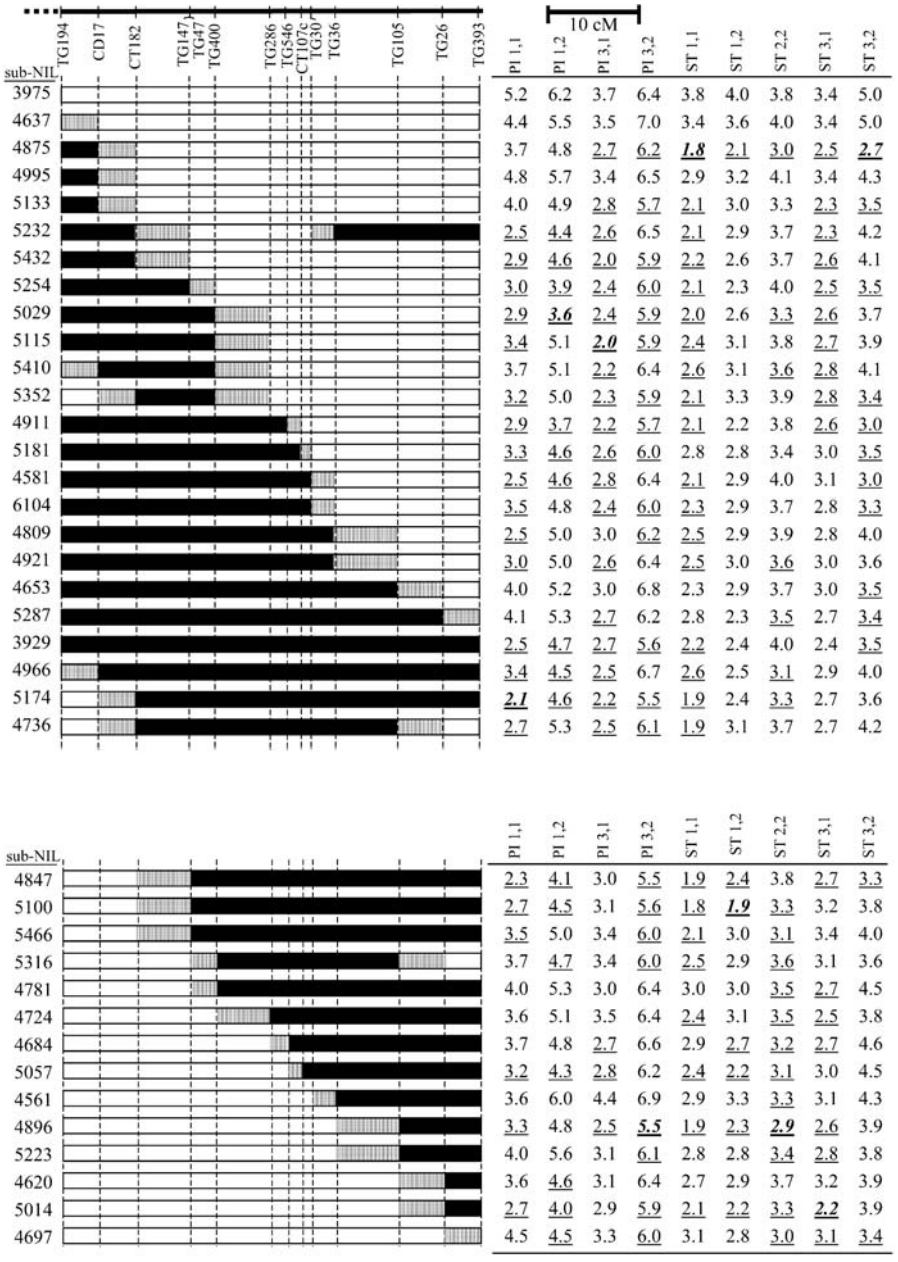

Fig. 3 Graphical genotypes and late blight resistance phenotype (*PI* and *ST*) means for sub-NILs covering the *L. hirsutum* introgression on NIL11. *Open bars* represent homozygous *L. esculentum*, *solid bars* represent homozygous *L. hirsutum*, and *gray bars* represent intervals containing crossovers. PI and ST were scored at three locations (1, 2, 3) on two dates (1, 2). Only LSMEANS from ANOVAs with significant genotype effects are shown for each sub-NIL. The most resistant sub-NIL in each column is shown in *bold*, and lines significantly different from the average of the susceptible controls (NC 84173 and Hypeel 45) are *underlined*

controls. Six-week-old seedlings were transplanted in late May at Salinas and at Majors and Swanton in mid-June. Each plot contained five plants spaced 0.61 m apart. Plots were separated by 1-m spacing between plots in a row and 1.21-m spacing between rows. No fungicides were applied, and plants were sprinkler-irrigated as needed. Standard horticultural practices were used; however, the Majors and Swanton locations were on organic farms, requiring the use of only organic soil amendments.

Horticultural traits (four vegetative and four reproductive) were evaluated by visual scoring at Salinas and Swanton. The traits were plant height (1 = 20–34 cm to 5 = >80 cm in 15-cm increments), plant shape (1 = very spreading to 5 = very upright), plant size (1 = very small to 5 = very large), canopy density (1 = very dense to 5 = very open), maturity (1 = flower buds only to 5 = large fruit), fruit yield (1 = no fruit to 5 = many fruit set), fruit size (1 = extra small, <1 cm in diameter, to 5 = extra large, >5 cm in diameter), and percent ripe (in 10% increments). Plant height, plant shape, plant size, canopy density, and maturity were scored in mid-August, and fruit yield, fruit size, and percent ripe were scored in mid-September. Control plots were used to calibrate scores between blocks and locations.

A natural *P. infestans* infection was detected at Majors in mid-August. This local isolate was propagated in the laboratory on leaves of susceptible NC 84173. Subsequently, each plot at all three sites was inoculated with a 1×10^3 spores ml^{-1} sporangial suspension of this isolate to encourage uniform disease development. The fields were sprinkler-irrigated to increase humidity prior to inoculation. The Majors location was inoculated one week after the initial infection was observed. The Salinas and Swanton sites were inoculated mid-September after the second horticultural trait assessment. Disease was assessed on two dates at each location, 8 and 16 days after infection was detected or after inoculation. Disease resistance was scored as percent infected leaf area (PI) on a plot basis using a 0–7 scale (modified from James 1971) as 0 = no infected leaves, 1 = up to two leaves per plant infected, 2 = 5–24% of leaf area infected (LAI), 3 = 25–49% of LAI, 4 = 50–79% of LAI, 5 = 80–94% of LAI, 6 = 95–99% of LAI, and 7 = 100% of LAI) and as stem resistance (ST) on a scale of 1–5 (1 = no stem

lesions, 2 = threadlike stem lesions, 3 = stem lesions <3 cm long; 4 = stem lesions >3 cm long, <50% stems with lesions, and 5 = >50% stems with lesions).

Data analysis and QTL mapping

Since comparisons among sub-NILs within a NIL set were desired, each set of sub-NILs was analyzed separately as a randomized complete block design using PROC GLM of SAS (SAS Institute 1996) to perform analysis of variance (ANOVA). Levene's test for homogeneity of variance indicated that horticultural traits could be analyzed across locations; however, neither PI nor ST data could be pooled across locations. With three locations scored on two dates, a total of six disease evaluations were analyzed for both PI and ST. Gradients in disease severity occurred at all sites due to prevailing environmental conditions, so the ANOVAs for PI and ST included a covariate to adjust for non-blocked variation in the intensity of *P. infestans* infection. The median of the eight plots adjacent to each plot was used as the covariate. The LSMEANS, which were adjusted for the covariate in the model, were used in subsequent analyses.

Fine mapping of QTLs for disease resistance and horticultural traits was performed by two methods. First, at each marker locus, phenotypic data for lines homozygous for cultivated *L. esculentum* (EE) or wild *L. hirsutum* (HH) alleles were grouped, and then the phenotypic means for the EE and HH genotype groups were compared using two-sample *t*-tests calculated by PROC GLM of SAS (SAS Institute 1996). The *t*-test *P*-values for marker genotype mean comparisons were graphed as $-\log(P)$; thus, the most likely QTL positions are indicated by peaks (Monforte and Tanksley 2000). Significance thresholds at $P = 0.05$, 0.01, and 0.001 were 1.3, 2.0, and 3.0, respectively. Secondly, evidence for the location of disease resistance QTLs was also obtained by identifying individual sub-NILs that were more resistant than the average of the two susceptible controls (NC 84173 and Hypeel 45) according to the least significant difference (LSD) at $P = 0.05$. Only PI and ST disease evaluations with a significant genotype effect in the ANOVA were presented for this analysis (see Figs. 1, 2, 3). Significance at the majority of the six location/date combinations scored by either PI or ST suggested the presence of a QTL in a sub-NIL. When several sub-NILs showed a QTL phenotypic effect, the QTL was deduced to be within the *L. hirsutum* interval shared by the sub-NILs. A $P \leq 0.05$ significance level was used to reduce the type I error in order to increase the probability of identifying all sub-NILs that exhibited resistance. The *P*-level was not adjusted for the multiple means tested because the likelihood of a sub-NIL being significantly resistant by chance alone in all or most of the location/date combinations is low. The additive effect at a QTL was estimated by (HH-EE)/2EE (Monforte and Tanksley 2000). The names of fine-mapped QTLs correspond to the names used on the BC_1 map (Brouwer et al., in press).

Results and discussion

NIL and sub-NIL line development

Individuals homozygous for *L. esculentum* alleles at all 32 background markers in each NIL were identified by sequential background selection in the BC_2, BC_3, and BC_4 generations. Fewer double recombinants were detected (0/519=0% for the 24-cM introgression in NIL5 and 15/428=3.6% for the 41-cM introgression in NIL11) compared to the expected 6% between markers separated by 25 cM, assuming no interference. The results suggest that background selection was effective in recovering the majority of the *L. esculentum* genome**.** On average, at least 75% of the background genome in the BC_4 NILs should be homozygous for Hypeel 45 alleles contributed by the BC_2 to BC_4 backcrosses. It was not possible to distinguish the NC 84173 allele from Hypeel 45 due to the lack of DNA polymorphisms between these cultivars. However, all heterozygous NILs must contain an intact *L. esculentum* segment at the target QTL from recurrent parent Hypeel 45. The use of large BC_5 mapping populations (504, 519, and 428 plants for NIL4, NIL5, and NIL11, respectively) ensured that a complete set of vigorous, highly fertile sub-NILs with overlapping *L. hirsutum* segments were obtained. Several sub-NILs were chosen when recombination breakpoints occurred in large intervals (>3 cM).

BC_5S_1 maps for QTL regions *lb4*, *lb5b*, and *lb11b* contained 8, 18, and 14 markers and covered 22.7, 24.7, and 40.8 cM for NIL4, NIL5, and NIL11, respectively (Figs. 1, 2, 3). The BC_5 fine-map distances for the NIL4, NIL5, and NIL11 intervals were reduced by 19%, 26%, and 14%, respectively, compared to distances in the BC_1 mapping population (Brouwer et al., in press). In our BC_1 map, the three QTL intervals showed suppressed recombination compared to other maps (56–69 cM in Pillen et al. 1996; 44–85 cM in Bernacchi and Tanksley 1997). Reduced recombination has been observed previously in introgressed intervals from wild tomato species (Rick 1969; Ganal and Tanksley 1991; Alpert and Tanksley 1996), but not in intraspecific NILs of maize (Graham et al. 1997) and rice (Wissuwa et al. 2002). Thus, reduced recombination in our NILs was probably a consequence of introducing wild-species DNA into a cultivated genetic background. Sequence divergence between wild and cultivated *Lycopersicon* species has been suggested as the cause of reduced recombination in interspecific populations (Paterson et al. 1990). If crossovers preferentially occurred in homozygous *L. esculentum* intervals, recombination suppression would be observed in regions heterozygous for the wild-species allele (Monforte and Tanksley 2000). Recombination suppression was more severe when the introgressed interval was smaller (i.e., NIL4 and NIL5 compared to NIL11). Similarly, an 80% reduction in recombination was observed for a *L. hirsutum* introgression covering a third of chromosome 1 (Monforte and Tanksley 2000) and a *L. pimpinellifolium* introgression covering a quarter of chromosome 8 (Ku et al. 2000).

Fine mapping in NIL4 sub-NILs

Marker genotype mean *t*-tests indicated that the resistance QTL *lb4* was located between TG182 and CT194, a 6.9-cM interval, and centered on TG609 for both PI and ST (Fig. 4, NIL4). Comparisons were significant at TG609 for four of six disease evaluations of PI and for five of six evaluations of ST (Fig 4, NIL4). The non-significant comparisons were all observed on the second evaluation date when advanced late blight disease limited the ability to discern phenotypic differences. The additive effect of

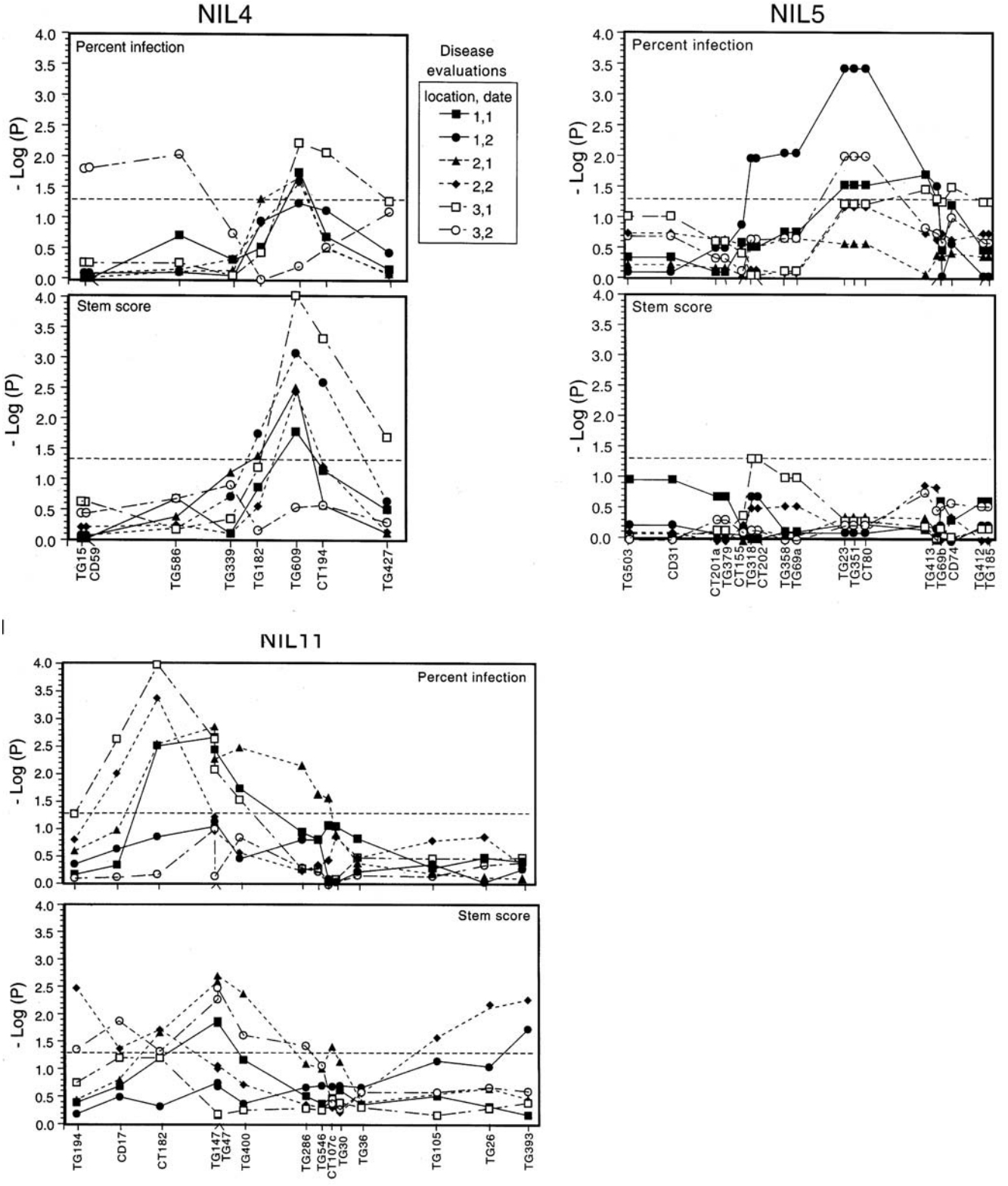

Fig. 4 Significance [–log(*P*)] of marker-genotype mean *t*-test comparisons across the NIL4, NIL5, and NIL11 introgressed regions. Both percent infection (*PI*) and stem score (*ST*) are shown for the six disease evaluations at three locations (1, 2, 3) scored on two dates (1, 2). Significance at $P<0.05$ is indicated by the *horizontal dashed line*

substituting *L. hirsutum* (H) alleles for *L. esculentum* (E) alleles at TG609 ranged from −0.44 to −0.67 for PI and from −0.44 to −0.98 for ST. The −log(*P*) values and additive effects were higher for ST, suggesting that *lb4* contributes more to stem resistance. The analysis of individual sub-NILs supported the presence of a single QTL near TG609. All sub-NILs with H alleles between TG609 and CT194 were more resistant than the controls in most disease evaluations (Fig. 1, sub-NIL 7842 down to sub-NIL 7620). Most sub-NILs with E alleles in this interval did not show resistant phenotypes. The exception was sub-NIL 7428, with an introgression between TG15 and TG586, which was consistently more resistant than the susceptible controls (Fig 1). Sub-NIL 7417, which contained the same interval, was not resistant. The resistance in 7428 is unlikely to indicate the presence of a second resistance locus within this region and instead may be due to environmental effects and/or residual *L. hirsutum* intervals in the genomic background (see "Comparison of fine mapping methods").

Lb4 was identified in the BC_1 mapping population by all three assay methods and for both stem and foliage resistance, but only in one experiment of each assay method (Brouwer et al., in press). In the BC_1, the peak LOD for one field experiment detecting *lb4* mapped to TG609, but peaks for the other two experiments were located outside of the interval identified for *lb4* by sub-NIL fine mapping. The effects of *lb4* may have been masked by segregation at other resistance QTLs and/or environmental variation in the BC_1 population. Resistance was expressed more consistently in the sub-NILs. The refined *lb4* interval is coincident with *Pi1*, a potato late blight quantitative resistance QTL first identified by Leonards-Schippers et al. (1994). This interval was associated with quantitative resistance in multiple mapping populations of potato, and explained large portions of the phenotypic variances (Collins et al. 1999; Gebhardt and Valkonen 2001). The *lb4* interval also coincides with a major resistance QTL region derived from *Solanum microdontum* (Sandbrink et al. 2000). However, *lb4* appears to map outside of a *Solanaceous* resistance gene cluster that contains *R2* for *P. infestans* resistance in potato and the tomato nematode resistance gene *Hero* (Grube et al. 2000); thus, *lb4* is unlikely to be an allelic variant of *R2*.

QTLs were detected for five horticultural traits in NIL4: plant shape, canopy density, maturity, fruit yield, and fruit size (Fig. 5, NIL4). Sub-NILs containing H alleles at these QTLs exhibited a more spreading habit, less dense canopy, and earlier maturity with undesirable reductions in yield and fruit size (data not shown). The canopy density peak mapped to the *lb4* interval centered on TG609, but maturity, fruit yield, and fruit size QTLs mapped adjacent to TG609, between CT194 and TG427. The −log(*P*) plots for the reproductive traits were very similar, suggesting pleiotropic effects of a single gene or tightly linked loci (Fig. 5). Our fruit size QTL may correspond to *fw4.1*, a major "domestication" QTL affecting fruit size in tomato located between TG339 and CT194 (Grandillo et al. 1999). Although several other studies examined horticultural traits including fruit yield and maturity, *fw4.1* was the only QTL coincident to our NIL4 intervals that contains both resistance and horticultural QTLs (Eshed and Zamir 1995; Fulton et al. 1997; Bernacchi et al. 1998b).

Fine mapping in NIL5 sub-NILs

Resistance QTL *lb5b* mapped to an 8.8-cM interval between TG69a and TG413, and the peak was in a 1.4-cM interval between TG23 and CT80. Marker genotype mean *t*-test comparisons were significant only for PI (Fig. 4, NIL5). Three out of six disease evaluations had significant peaks in this interval and two others were just below the significance threshold. The additive effect of substituting H alleles for E alleles at TG23 ranged from −0.41 to −0.91 for PI. Most sub-NILs that contained H alleles between TG23 and CT80 (Fig. 2, sub-NIL 10052 through sub-NIL 10607) were significantly more resistant than the susceptible controls in at least three of the four assays, concurring with the genotype mean *t*-tests (Fig. 4, NIL5). The lone exception was sub-NIL 3681, which was significant in only one of the four assays. All sub-NILs with E alleles between TG23 and CT80 and H alleles between CT80 and TG185 (Fig. 2, sub-NIL 10670 through sub-NIL 10747) were significantly more resistant in half of the resistance evaluations, hinting at the presence of another resistance locus between CT80 and TG185, an 8.4-cM interval (Fig. 2). However, the genotype means comparisons did not clearly indicate a separate peak between CT80 and TG185 (Fig. 4, NIL5).

In the BC_1 mapping study, *lb5b* was identified by all assay methods and was the most consistently detected resistance QTL (Brouwer et al., in press). The peak LOD score of *lb5b* mapped within 5 cM of TG23 in the BC_1 population, in agreement with the location identified here by sub-NIL fine mapping. Resistance at *lb5b* appears to be foliage-specific, since resistance QTLs in both studies were identified only for traits measuring resistance on leaves. The refined *lb5b* interval does not coincide with the *Solanaceous* resistance gene cluster on chromosome 5 that contains *Pto* (near TG379, Grube et al. 2000), as originally suggested by our BC_1 data (Brouwer et al., in press). However, *lb5b* does co-localize with *Rx-3* for resistance to *Xanthomonas campestris* pv. *vesicatoria* in tomato and *Gro*VI for nematode resistance in potato (Grube et al. 2000).

QTLs were detected in NIL5 for plant shape and reproductive traits (Fig 5, NIL5). Sub-NILs containing H alleles were earlier flowering, had undesirable reductions in yield and fruit size, and had fewer ripe fruit (data not shown). The two QTLs detected for plant shape were barely significant and mapped between TG503 and CT201a and TG69b and TG185. QTLs for maturity, fruit yield, and fruit size all mapped between TG379 and TG69a, although the marker with the peak −log(*P*) value varied. The QTL for percent ripe fruit mapped between

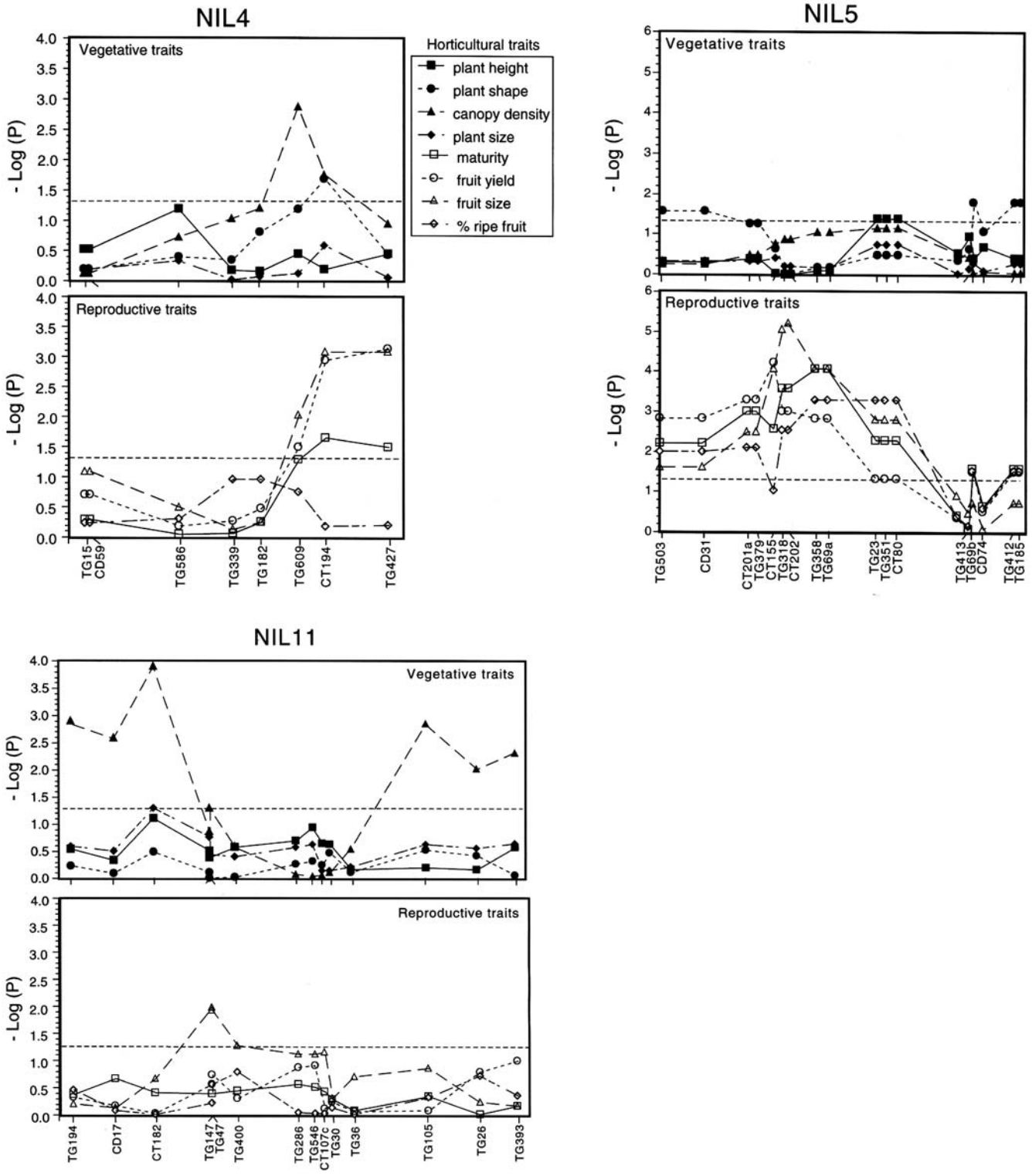

Fig. 5 Significance [−log(*P*)] of marker-genotype mean *t*-test comparisons across the NIL4, NIL5, and NIL11 introgressed regions for eight horticultural traits. Significance at $P<0.05$ is indicated by the *horizontal dashed line*

CT202 and CT80. The overlapping QTL position for all reproductive traits suggests that a single gene or gene cluster may affect all four traits, or alternatively, linked loci affect each trait separately. QTLs for horticultural traits were not identified previously in the *lb5b* interval (Fulton et al. 1997; Bernacchi et al. 1998b; Grandillo et al. 1999), although QTLs for red fruit yield and maturity were mapped near TG503 in a population from *L.*

peruvianum (Fulton et al. 1997). The QTLs for plant shape, maturity, yield, and fruit size map to intervals adjacent to, but not overlapping with, *lb5b*.

Fine mapping in NIL11 sub-NILs

Resistance QTL *lb11b* was located between TG194 and TG400 for both PI and ST, a 15.1-cM interval (Fig. 4, NIL11). Based on the peaks of $-\log(P)$ values, the most likely position of the QTL contributing to both PI and ST is located in the 5.2-cM interval between CT182 and TG147. The $-\log(P)$ values were higher for PI, suggesting that *lb11b* contributes more to foliage resistance. The additive effect of substituting H for E alleles at TG147 ranged from −0.32 to −0.67 for PI and from −0.21 to −0.44 for ST. The relative size of the additive effects for *lb4*, *lb5b*, and *lb11b* suggest that *lb5b* confers the most to foliage resistance (PI) while *lb4* contributes the most to stem resistance (ST).

A second resistance QTL, designated *lb11c* to distinguish it from *lb11b*, was present in NIL11 near TG393 (Fig. 4, NIL11). *Lb11c* was detected only for ST on the second scoring date. Individual sub-NILs containing H alleles at *lb11b* (Fig. 3, sub-NIL 5432 through sub-NIL 5466) were more resistant than the susceptible controls in most evaluations. However, many sub-NILs with E alleles in the *lb11b* interval and H alleles in the TG105-TG393 interval were also more resistant than the susceptible controls in most evaluations, supporting the presence of *lb11c* in NIL11 (Fig. 3).

In the BC_1 mapping study, resistance at *lb11b* was identified only in field assays (Brouwer et al., in press). Peak LOD values of QTLs for two of three resistance traits mapped near marker TG147, while the peak LOD for the third trait mapped near TG393, providing further evidence for the presence of two QTLs in the NIL11 introgressed interval. QTL were only detected for PI in the BC_1, but the sub-NILs have clearly shown that these QTLs contribute to both stem and foliage resistance. *Lb11b* did not coincide with any previously mapped resistance genes, but *Lb11c* maps to the tomato resistance gene cluster located near TG393 at the end of chromosome 11 (Grube et al. 2000). This diverse gene cluster contains resistance genes against viruses, nematodes, and fungi, including *R3*, *R6*, and *R7* in potato for qualitative resistance to late blight (Grube et al. 2000).

QTLs were detected for two horticultural traits in NIL11: canopy density and fruit size (Fig. 5, NIL11). Two canopy-density QTLs were mapped to locations coincident with *lb11b* and *lb11c*. At both QTLs, more open canopies were associated with increased disease resistance. A fruit size QTL was detected between TG147 and TG400 (Fig. 5, NIL11), coincident with *lb11b*. *L. hirsutum* alleles were associated with reduced fruit weight at this QTL (data not shown). QTLs for yield and fruit weight were identified near TG147 in a population from *L. hirsutum* (Bernacchi et al. 1998b), but QTLs associated with horticultural traits in the *lb11b* interval were not detected in other interspecific tomato populations (Fulton et al. 1997; Grandillo et al. 1999).

Comparison of fine mapping methods

The first method compared the marker genotype means using *t*-tests at each marker locus across the introgressed interval and is analogous to the QTL mapping methods used to identify QTLs in early generation populations. Identical results for fine mapping *lb11b* and *lb11c* were obtained by *t*-tests and by composite interval mapping using the selected sub-NILs and the BC_5 map (data not shown). However, as with interval mapping, genotype mean comparisons also may fail to separate closely linked QTLs and detect QTLs linked in repulsion. The averaging of genotypes with large introgressions lessens the ability to locate a QTL as precisely as in the second method, termed substitution mapping by Paterson et al. (1990). In substitution mapping, the genotypes and trait phenotypes of individual sub-NILs are compared with susceptible controls to identify sub-NILs containing genetic factors contributing to specific traits. By comparing sub-NILs with overlapping introgressed regions, chromosome intervals likely to contain relevant genes can be identified and precisely located. Substitution mapping has been very successful in identifying genes with large phenotypic effects in many species, including tomato (Ku et al. 2000; Fridman et al. 2002). Substitution mapping alone was not sufficient to fine map our resistance QTLs associated with more moderate phenotypic effects. Our sub-NIL phenotypes were not always consistent across all disease evaluations, and resistance scores in a few sub-NILs were much higher or lower than expected based on the genotype of the sub-NIL (e.g., 3681 not as resistant as expected in NIL5). The unexpected scores confuse the substitution mapping analysis and can indicate environmental variation effects on the resistance phenotype, residual segregating background *L. hirsutum* intervals that remained despite background MAS, and/or influence of *L. esculentum* genomic background on the expression of disease resistance QTLs (Robert et al. 2001). In our study, all sub-NILs in a NIL set were derived from the same BC_4 plant, which should minimize the heterogeneity in the genetic background. By using both analyses, the intervals identified by genotype mean comparisons could be confirmed and refined using substitution mapping.

Breeding and selection implications

QTL effects identified in early generation mapping populations may inaccurately estimate the map position and size of QTL effects and have limited ability to distinguish linkage from pleiotropy (Kearsey and Farquhar 1998; Utz et al. 2000). Due to the limitations of early generation mapping, Young (1999) suggested that QTL effects be confirmed by evaluation over multiple locations, in unrelated genomic backgrounds, and fine-mapped in near-

isogenic lines before introgression into elite germplasm. Wild species of tomato have undesirable alleles at many loci associated with horticultural traits that are important in the domestication and cultivation of tomato (Tanksley et al. 1996; Bernacchi and Tanksley 1997; Bernacchi et al. 1998a; Grandillo et al. 1998). The utilization of quantitative disease resistance genes derived from wild tomato species can be limited by linkage drag caused by undesirable alleles at horticulturally important loci.

In this study, we verified the effects and refined the map position of three QTLs for quantitative resistance to late blight in tomato. Quantitative resistance was conferred by *L. hirsutum* alleles at all three QTLs despite being introgressed into a different *L. esculentum* background. The subsequent identification of small marker intervals flanking the resistance QTLs provide interesting targets for marker-assisted introgression into elite tomato cultivars. MAS would be particularly advantageous in pyramiding the quantitative resistance QTLs into a single genotype. If the resistance alleles at the three QTLs work additively in conferring resistance to late blight, the combined additive effect of reducing percent infection by −2.0 and stem scores by −1.3 could provide significant improvement in late blight resistance in cultivated tomato. In a separate study, the full BC_1 intervals containing the four resistance QTLs (*lb3*, *lb4*, *lb5b*, and *lb11b*) were simultaneously introgressed into cultivated tomato and evaluated in homozygous combinations of the four resistance loci (D.J. Brouwer, S.K. Coulibaly, and D.A. St. Clair, unpublished data). Lines containing more than one QTL interval were more resistant than lines containing a single QTL interval, and resistance in lines with multiple QTLs was also less environmentally variable. Unfortunately, lines with all four QTLs were also horticulturally undesirable. Our study here indicated that the fine-mapped intervals containing resistance QTLs *lb4*, *lb5b*, and *lb11b* do not contain genes conferring major deleterious horticultural effects, although some linked intervals did contain undesirable *L. hirsutum* alleles for horticultural traits. Classical backcross breeding might be used to introgress quantitative resistance to late blight from LA2099 into cultivated tomato. However, the close linkage of *lb4* and *lb5b* with deleterious H alleles at QTLs associated with horticultural traits suggests that identification of favorable recombinants would be very challenging without molecular marker information.

Acknowledgements This research was supported by a USDA-NRI Plant Genome Program, grant 00-35300-9413, to D.A.S. and the California Tomato Commission. The authors thank the USDA station in Salinas, Calif.; Swanton Berry Farm in Majors, Calif; and Jacobs Farm in Swanton, Calif. for field locations and assistance.

References

Alpert KB, Tanksley SD (1996) High-resolution mapping and isolation of a yeast artificial chromosome contig containing *fw2.2* - a major fruit weight quantitative trait locus in tomato. Proc Natl Acad Sci USA 93:15503–15507

Bernacchi D, Tanksley SD (1997) An interspecific backcross of *Lycopersicon esculentum* × *L. hirsutum*: linkage analysis and a QTL study of sexual compatibility factors and floral traits. Genetics 147:861–877

Bernacchi D, Beck-Bunn T, Emmatty D, Eshed Y, Inai S, Lopez J, Petiard V, Sayama H, Uhlig J, Zamir D, Tanksley S (1998a) Advanced backcross QTL analysis of tomato. II. Evaluation of near-isogenic lines carrying single-donor introgressions for desirable wild QTL-alleles derived from *Lycopersicon hirsutum* and *L. pimpinellifolium*. Theor Appl Genet 97:1191–1196

Bernacchi D, Beck-Bunn T, Eshed Y, Lopez J, Petiard V, Uhlig, J, Zamir D, Tanksley S (1998b) Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon hirsutum*. Theor Appl Genet 97:381–397

Brouwer DJ, Jones ES, St. Clair DA (2003) QTL analysis of quantitative resistance to *Phytophthora infestans* (late blight) in tomato, and comparisons to potato. Genome (in press)

Chunwongse J, Chunwongse C, Black LL, Hanson PM (1998) Mapping of the *Ph3* gene for late blight from *L. pimpinellifolium* L3708. Rep Tomato Genet Coop 48:963–971

Collins A, Milbourne D, Ramsay L, Meyer R, Chatot-Balandras C, Oberhagemann P, De Jong W, Gebhardt C, Bonnel E, Waugh R (1999) QTL for field resistance to late blight in potato are strongly correlated with maturity and vigour. Mol Breed 5:387–398

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141:1147–1162

Ewing EE, Simko I, Smart CD, Bonierbale MW, Mizubuti ESG, May GD, Fry WE (2000) Genetic mapping from field tests of qualitative and quantitative resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. Mol Breed 6:25–36

Fridman E, Liu YS, Carmel-Goren L, Gur A, Shoresh M, Pleban T, Eshed Y, Zamir D (2002) Two tightly linked QTLs modify tomato sugar content via different physiological pathways. Mol Genet Genomics 266:821–826

Fry WE, Goodwin SB (1997) Re-emergence of potato and tomato late blight in the United States. Plant Dis 81:1349–1357

Fulton TM, Chunwongse J, Tanksley SD (1995) Microprep protocol for the extraction of DNA from tomato and other herbaceous plants. Plant Mol Biol Rep 13:207–209

Fulton TM, Beck-Bunn T, Emmatty D, Eshed Y, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley SD (1997) QTL analysis of an advanced backcross of *Lycopersicon peruvianum* to the cultivated tomato and comparisons with QTLs found in other wild species. Theor Appl Genet 95:881–894

Gallegly ME (1960) Resistance to the late blight fungus in tomato. Proceedings Plant Science Seminar. Campbell Soup Co., Camden, N.J., p 113–135

Ganal MW, Tanksley SD (1991) Recombination around the *Tm2a* and *Mi* resistance genes in different crosses of *Lycopersicon pimpinellifolium*. Theor Appl Genet 92:935–951

Gardner RG (1992) Mountain Spring NC 8276 and NC 84173 tomato breeding lines. Hortscience 27:1233–1234

Gebhardt C, Valkonen JPT (2001) Organization of genes controlling disease resistance in the potato genome. Annu Rev Phytopathol 39:79–102

Graham GI, Wolff DW, Stuber CW (1997) Characterization of a yield quantitative trait locus on chromosome five of maize by fine mapping. Crop Sci 37:1601–1610

Grandillo S, Ku HM, Tanksley SD (1999) Identifying the loci responsible for natural variation in fruit size and shape in tomato. Theor Appl Genet 99:978–987

Grube RC, Radwanski ER, Jahn M (2000) Comparative genetics of disease resistance within the Solanaceae. Genetics 155:873–887

James C (1971) A manual of assessment keys for plant diseases. Canada Department of Agriculture. Publication No. 1458

Kearsey MJ, Farquhar AGL (1998) QTL analysis in plants—where are we now. Heredity 80:137–142

Konieczny A, Ausubel FM (1993) A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4:403-410

Ku HM, Grandillo S, Tanksley SD (2000) *fs8.1*, a major QTL, sets the pattern of tomato carpel shape well before anthesis. Theor Appl Genet 101:873–878

Kuhl JC, Hanneman RE, Havey MJ (2001) Characterization and mapping of *Rpi1*, a late-blight resistance locus from diploid (1EBN) Mexican *Solanum pinnatisectum*. Mol Genet Genomics 265:977–985

Leonards-Schippers C, Gieffers W, Schaferpregl R, Ritter E, Knapp SJ, Salamini F, Gebhardt C (1994) Quantitative resistance to *Phytophthora infestans* in potato—a case study for QTL mapping in an allogamous plant species. Genetics 137:67–77

Lincoln S, Daly M, Lander ES (1992) Constructing genetic maps with MAPMAKER/EXP 3.0, 3rd edn. The Whitehead Institute Technical Report

Monforte AJ, Tanksley SD (2000) Fine mapping of a quantitative trait locus (QTL) from *Lycopersicon hirsutum* chromosome 1 affecting fruit characteristics and agronomic traits: breaking linkage among QTLs affecting different traits and dissection of heterosis for yield. Theor Appl Genet 100:471–479

Moreau P, Thoquet P, Olivier J, Laterrot H, Grimsley N (1998) Genetic mapping of Ph-2, a single locus controlling partial resistance to *Phytophthora infestans* in tomato. Mol Plant-Microbe Interact 11:259–269

Naess SK, Bradeen JM, Wielgus SM, Haberlach GT, McGrath JM, Helgeson JP (2000) Resistance to late blight in *Solanum bulbocastanum* is mapped to chromosome 8. Theor Appl Genet 101:697–704

Oberhagemann P, Chatot-Balandras C, Schafer-Pregl R, Wegener D, Palomino C, Salamini F, Bonnel E, Gebhardt C (1999) A genetic analysis of quantitative resistance to late blight in potato: towards marker-assisted selection. Mol Breed 5:399–415

Paterson AH, De-Verna JW, Lanini B, Tanksley SD (1990) Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes, in an interspecific cross of tomato. Genetics 124:735–742

Pillen K, Pineda O, Lewis CB, Tanksley SD (1996) Status of genome mapping tools in the taxon Solanaceae. In: Paterson AH (ed) Genome mapping in plants. Landes, Austin, Tex., pp 281–307

Rick CM (1969) Controlled introgression of chromosomes from *Solanum pennellii* into *Lycopersicion esculentum*: segregation and recombination. Genetics 62:753–768

Robert VJM, West MAL, Inai S, Caines A, Arntzen L, Smith JK, St Clair DA (2001) Marker-assisted introgression of blackmold resistance QTL alleles from wild *Lycopersicon cheesmanii* to cultivated tomato (*L. esculentum*) and evaluation of QTL phenotypic effects. Mol Breed 8:217–233

Saito K, Miura K, Nagano K, Hayano-Saito Y, Araki H, Kato A (2001) Identification of two closely linked quantitative trait loci for cold tolerance on chromosome 4 of rice and their association with anther length. Theor Appl Genet 103:862–868

Sandbrink JM, Colon LT, Wolters P, Stiekema WJ (2000) Two related genotypes of *Solanum microdontum* carry different segregating alleles for field resistance to *Phytophthora infestans*. Mol Breed 6:215–225

SAS Institute I (1996) The SAS system for Windows. SAS Institute, Cary, N.C.

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis—a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191–203

Truco MJ, Randall LB, Bloom AJ, St. Clair DA (2000) Detection of QTLs associated with shoot wilting and root ammonium uptake under chilling temperatures in an interspecific backcross population from *Lycopersicon hirsutum* × *L. hirsutum*. Theor Appl Genet 101:1082–1092

Utz HF, Melchinger AE, Schon CC (2000) Bias and sampling error of the estimated proportion genotypic variance explained by quantitative trait loci determined from experimental data in maize using cross validation and validation with independent samples. Genetics 154:1839–1849

Visker M, Keizer LCP, Van Eck HJ, Jacobsen E, Colon LT, Struik PC (2003) Can the QTL for late blight resistance on potato chromosome 5 be attributed to foliage maturity type? Theor Appl Genet 106:317–325

Wastie RL (1991) Breeding for resistance. In: Ingram DS, Williams PH (eds) Advances in plant pathology. Academic Press, London, pp 193–224

Wissuwa M, Wegner J, Ae N, Yano M (2002) Substitution mapping of *Pup1*: a major QTL increasing phosphorus uptake of rice from a phosphorus-deficient soil. Theor Appl Genet 105:890–897

Yamamoto T, Kuboki Y, Lin SY, Sasaki T, Yano M (1998) Fine mapping of quantitative trait loci *Hd-1*, *Hd-2* and *Hd-3*, controlling heading date of rice, as single Mendelian factors. Theor Appl Genet 97:37–44

Young ND (1999) A cautiously optimistic vision for marker-assisted breeding. Mol Breed 5:505–510