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Identification of QTLs for early blight (*Alternaria solani*) resistance in tomato using backcross populations of a *Lycopersicon esculentum* × *L. hirsutum* cross

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Abstract Most commercial cultivars of tomato, *Lycopersicon esculentum* Mill., are susceptible to early blight (EB), a devastating fungal (*Alternaria solani* Sorauer) disease of tomato in the northern and eastern parts of the U.S. and elsewhere in the world. The disease causes plant defoliation, which reduces yield and fruit quality, and contributes to significant crop loss. Sources of resistance have been identified within related wild species of tomato. The purpose of this study was to identify and validate quantitative trait loci (QTLs) for EB resistance in backcross populations of a cross between a susceptible tomato breeding line (NC84173; maternal and recurrent parent) and a resistant *Lycopersicon hirsutum* Humb. and Bonpl. accession (PI126445). Sixteen hundred BC_1 plants were grown to maturity in a field in 1998. Plants that were self-incompatible, indeterminant in growth habit, and/or extremely late in maturity, were discarded in order to eliminate confounding effects of these factors on disease evaluation, QTL mapping, and future breeding research. The remaining 145 plants (referred to as the BC_1 population) were genotyped for 141 restriction fragment length polymorphism (RFLP) markers and 23 resistance gene analogs (RGAs), and a genetic linkage map was constructed. BC_1 plants were evaluated for disease symptoms throughout the season, and the area under the disease progress curve (AUDPC) and the final percent defoliation (disease severity) were determined for each plant. BC_1 plants were self-pollinated and produced BC_1S_1 seed. The BC_1S_1 population, consisting of 145 BC_1S_1 families, was grown and evaluated for disease symptoms in replicated field trials in two subsequent years (1999 and 2000) and AUDPC and/or final percent defoliation were determined for each family in each year. Two QTL mapping approaches, simple interval mapping (SIM) and composite interval mapping (CIM), were used to identify QTLs for EB resistance in the BC_1 and BC_1S_1 populations. QTL results were highly consistent across generations, years and mapping approaches. Approximately ten significant QTLs (LOD ≥ 2.4, $P \leq 0.001$) were identified (and validated) for EB resistance, with individual effects ranging from 8.4% to 25.9% and with combined effects of >57% of the total phenotypic variation. All QTLs had the positive alleles from the disease-resistant parent. The good agreement between results of the BC_1 and 2 years of the BC_1S_1 generations indicated the stability of the identified QTLs and their potential usefulness for improving tomato EB resistance using marker-assisted selection (MAS). Further inspections using SIM and CIM indicated that six of the ten QTLs had independent additive effects and together could account for up to 56.4% of the total phenotypic variation. These complementary QTLs, which were identified in two generations and 3 years, should be the most useful QTLs for MAS and improvement of tomato EB resistance using PI126445 as a gene resource. Furthermore, the chromosomal locations of 10 of the 23 RGAs coincided with the locations of three QTLs, suggesting possible involvement of these RGAs with EB resistance and a potential for identifying and cloning genes which confer EB resistance in tomato.

Keywords Composite interval mapping · resistance gene analogs (RGAs) · restriction fragment length polymorphism (RFLP) · self-incompatibility (SI) · QTL mapping

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Introduction

Early blight (EB), caused by the fungus *Alternaria solani* Sorauer, is one of the most common and destructive diseases of tomato, *Lycopersicon esculentum* Mill., in areas of heavy dew, frequent rainfall, and high relative humidity (RH); it can also be important in semi-arid areas when nightly dew is frequent (Frey and Horner 1957;

Dudley and Moll 1969; Falconer 1989). In the U.S., the disease can be severe in the Midwest, East and Northeast. Early blight is a 3-phased disease that can produce collar rot, leaf blight (early blight) and fruit rot. Collar rot has serious implications for tomato growers both as a disease and as a source of inoculum for an EB epidemic. The leaf blight phase, commonly referred to as early blight, is characterized by the formation of dark-colored spots that are necrotic in the center and result in a concentric ring pattern. As lesions expand and become more numerous, leaves are blighted and plants are gradually defoliated. Defoliation, which reduces yield and fruit quality and contributes to significant crop loss, is the most important phase of the disease. The calyx and fruit tissues are also susceptible to the fungus and, when infected, they contribute to reduced fruit yield and quality.

No genetic source of EB resistance is known within the cultivated species of tomato (Martin and Hepperly 1987; Foolad et al. 2000). However, resistant accessions have been identified within related wild species of tomato, in particular the green-fruited species *Lycopersicon hirsutum* Humb. and Bonpl. (Barksdale and Stoner 1977; Martin and Hepperly 1987; Nash and Gardner 1988a; Maiero et al. 1989) and the red-fruited species *Lycopersicon pimpinellifolium* (Jusl.) Mill. (Martin and Hepperly 1987; Kalloo and Banerjee 1993) (M.R. Foolad, unpublished data). Some resistant wild accessions have been utilized in traditional breeding programs and several breeding lines and cultivars with measurable levels of resistance have been developed (Barksdale and Stoner 1973; Gardner 1988; Nash and Gardner 1988b; Gardner and Shoemaker 1999). For example, several resistant breeding lines (Gardner 1988; Nash and Gardner 1988b) and a hybrid cultivar (Mountain Supreme) (Gardner and Shoemaker 1999) have been developed at the North Carolina State University. Resistant lines and cultivars can tolerate an extended fungicide spray interval and may contribute to a significant reduction in chemical inputs for EB control in tomato (Gardner 1988; Gardner and Shoemaker 1999). However, many resistant lines and cultivars are late maturing or low yielding, and/or the level of resistance is insufficient under EB epiphytotic field conditions (Maiero et al. 1989; Foolad et al. 2000). EB is associated with physiological maturity and fruit load of the plant. Late-maturing and/or low yielding plants appear resistant, while they may not possess genetic resistance. Such confounding factors together with the complex genetic nature of the resistance (Barksdale and Stoner 1977; Martin and Hepperly 1987; Gardner 1988; Nash and Gardner 1988a; Maiero et al. 1990) have contributed to a limited success in breeding for EB resistance using traditional approaches. At present, sanitation, long crop rotation, and routine application of fungicides are the most common control measures for EB disease. Thus, tomato breeders are still seeking EB-resistant cultivars with early to mid-season maturity and high yield potential. New strategies are needed for the identification, validation and effective transfer of genes for EB resistance from wild species into the cultivated tomato.

Molecular markers and maps and marker-assisted selection (MAS) technology is an alternative approach to traditional protocols of plant genetics and breeding. Molecular marker technology can facilitate precise determination of the number, chromosomal location, and individual and interactive effects of genes [or quantitative trait loci (QTLs)] that control complex traits. Following their identification, desirable QTLs can be introgressed into the cultigen and undesirable characteristics can be eliminated relatively rapidly by MAS (Tanksley et al. 1989). During the past several years, molecular maps have been developed for many plant species and successfully used for various genetic and breeding applications, including gene tagging and QTL mapping (Paterson et al. 1988; Foolad et al. 1997), MAS and germplasm development (Han et al. 1997; Stuber 1997; Toojinda et al. 1998; van-Berloo and Stam 1999) and map-based gene cloning (Martin et al. 1993; Brommenschenkel and Tanksley 1997). Furthermore, marker technology can facilitate exploitation of quantitative genetic variation within exotic germplasms and extend the genetic basis of the cultigen.

To expedite breeding for EB resistance in tomato using MAS technology, we have established a project to discern the genetic basis of EB resistance, including identification, mapping and characterization of QTLs for resistance in wild species of tomato. This paper reports the identification and validation of several QTLs for EB resistance in backcross populations of a cross between a resistant accession (PI126445) of *L. hirsutum* and a susceptible cultivated tomato line (NC84173).

Materials and methods

Plant material

Hybridizations were made between plants of a *L. esculentum* (hereafter referred to as E) breeding line (NC84173; pistillate parent) and a single plant of the *L. hirsutum* (hereafter referred to as H) accession PI126445. Original seed of NC84173 and PI126445 were obtained from R.G. Gardner, North Carolina State University, Fletcher, N.C., and the U.S. Department of Agriculture, Plant Genetic Resources Unit at Geneva, N.Y., respectively. NC84173 is a horticulturally superior, advanced breeding line (PVP) with a determinant (*spsp*) growth habit, mid-season maturity, and susceptibility to EB (R.G. Gardner, personal communication) (Foolad et al. 2000). PI126445 is a self-incompatible accession with an indeterminant (sp^+sp^+) growth habit, a vigorous vine and extremely late maturity that was previously identified to be highly resistant to tomato EB (Gardner 1988; Foolad et al. 2000). PI126445 is only unilaterally compatible (as pollen parent) in crosses with the cultivated tomato. A single F_1 hybrid plant was used as the pollen parent to hybridize plants of NC84173 and produced BC_1 seeds. The BC_1 population was used for marker analysis and map construction. The BC_1 and BC_1S_1 (self-pollinated progeny of BC_1) progeny were used for trait evaluation and QTL mapping.

Inoculum preparation

Two isolates (*126* and *134*) of the fungus *A. solani* Sorauer, previously obtained from naturally infected tomato plants in Pennsylvania, were used in this study. The cultures were grown on V-8 agar medium (17.7% V-8 juice, 0.3% $CaCO_3$, 2% agar) in 9-cm Petri

plates and incubated at 21–23 °C under cool-white fluorescent lamps with a 12-h photoperiod. After 10–14 days, conidia were harvested by flooding the plates with dH_2O (containing 0.01% of surfactant Tween 20, Fisher Scientific) and brushing the agar surface with a paintbrush. The spore concentration in the suspension medium was measured using a hemacytometer, and it was adjusted to approximately 2×10^4–3×10^4 conidia ml^{-1} before inoculation.

Trait evaluation

Screening of the BC_1 population

On June 5, 1998, 1,600 6-week-old greenhouse-grown BC_1 seedlings were transplanted into a field at the Pennsylvania State University Horticulture Research Farm, Rock Springs, Pa. (hereafter referred to as Penn State Research Farm). During the season, the BC_1 plants were examined for self-compatibility, growth habit and earliness in maturity. To eliminate confounding effects of these factors on disease evaluation, QTL identification and future breeding research, plants that were self-incompatible, indeterminant, and/or extremely late maturing were discarded and 145 plants that were self-compatible (as determined by the fruit load resulting from self-pollination), determinant, and early to mid-season maturing were retained for further studies (see R&D for justification). The field site was chosen for its proximity to natural sources of an inoculum of *A. solani*. However, to ensure a uniform source of the fungus, on August 12 the 145 BC_1 plants were spray-inoculated with a spore suspension mixture of the two *A. solani* isolates. Starting on August 25, and at approximately 7-day intervals thereafter, plants were evaluated for EB symptoms four times. At each evaluation, each plant was rated for EB symptoms using a modified Horsfall-Barratt rating scheme (Horsfall and Barratt 1945), with 0 indicating no visible symptom of EB infection and 100 indicating complete defoliation. A single rating was assigned to each plant at each evaluation. The final evaluation rating was considered as the final percent defoliation (disease severity). At the end of the season, fruits were harvested from individual BC_1 plants and processed to extract BC_1S_1 seeds.

Screening of the BC_1S_1 population in 1999

On June 3, 1999, 6-week-old greenhouse-grown seedlings of the 145 BC_1S_1 families (hereafter referred to as BC_1S_1-1999) and the two parents (NC84173 and PI126445) and asexual propagants of the original F_1 plant were transplanted into a field at the Penn State Research Farm. Plants were grown in a randomized complete block design (RCBD) with three blocks. Each block contained a 10-plant single-row plot of each of the 145 BC_1S_1 families and the F_1 generation and five 10-plant single-row plots of each of the two parents. The field site was chosen for its proximity to natural sources of inoculum of *A. solani*. However, to ensure a uniform source of the fungus, one plant of each of the susceptible cultivar New Yorker and breeding line NC84173 was planted at each end of each row plot to serve as a disease spreader and which were spray inoculated with a spore suspension mixture of the two *A. solani* isolates. Plants were rated for EB symptoms twice during the growing season, on August 25 and September 17. At each evaluation, plants were visually rated for % foliar defoliation, similar to that described for the BC_1 population, and a single rating was assigned to each 10-plant row plot (i.e., one rating for each plot). The second rating was considered as the final percent defoliation. During the season, the BC_1S_1 family plots were also examined and scored for earliness-in-maturity, as the time to 50% ripe fruit.

Screening of the BC_1S_1 population in 2000

A different sample of the same BC_1S_1 population (i.e., 145 BC_1S_1 families) was used for the experiment in 2000 (hereafter referred to as BC_1S_1-2000). The experiment was conducted similar to that in 1999, except that only two blocks were used and plants were evaluated for EB symptoms (final percent defoliation) only once on September 28.

Calculation of the area under the disease progress area

For each parental line (P_1 and P_2), BC_1 plant and BC_1S_1-1999 family, the area under the disease progress curve (AUDPC) was calculated as $\text{AUDPC} = \sum_{i=1}^{n} [(R_{i+1} + R_i)/2][t_{i+1} - t_i]$, where R = rating (estimated proportion of defoliated tissue) at the ith observation t_i = time (days) since previous rating at the ith observation, and n = total number of observations (Tooley and Grau 1984). For the parental lines and BC_1S_1-1999 progeny, the AUDPC was first calculated for each 10-plant plot and then averaged over replications. The AUDPC values and the final percent defoliation for the BC_1 plants and BC_1S_1 families were used as measures of resistance and for QTL identification (described below).

Marker analysis, map construction and QTL identification

RFLP analysis

Nuclear DNA was extracted from leaf tissues of the 145 BC_1 plants, using standard protocols for tomato (Bernatzky and Tanksley 1986; Foolad et al. 1997). DNAs were treated with RNAse and digested with five restriction enzymes, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I, according to the manufacturer's instructions (Amersham Pharmacia Biotech, N.J., USA). Agarose-gel electrophoresis, Southern blotting, hybridizations and autoradiography were as described elsewhere (Foolad et al. 1997). The DNA probes included 126 random tomato genomic (TG) or cDNA (CD or CT) clones obtained from S.D. Tanksley, Cornell University, Ithaca, N.Y., and 15 tomato cDNA clones obtained from K.J. Bradford, University of California, Davis, Calif.. The former clones were chosen based on the high-density molecular map of tomato (Pillen et al. 1996) so as to provide a uniform coverage of the genome. Probes were labeled with ^{32}P-dCTP by primer extension (Feinberg and Vogelstein 1983).

Resistance gene analog analysis

Sixteen oligonucleotide primers (eight pairs), previously designed based on the conserved leucine-rich repeat (LRR), nucleotide binding site (NBS) and serine/threonine protein kinase (PtoKin) motifs of several known resistance genes (Table 1), and standard PCR conditions (Foolad et al. 1995) were used for the amplification of resistance gene analogs (RGAs). Briefly, each PCR reaction was performed in a 25-µl volume consisting of 300 µM each of dATP, dCTP, dGTP and dTTP, 5 mM of $MgCl_2$, one unit of *Taq* DNA polymerase, 2.5 µl of 10 × buffer (PCR Core System I; Promega, Madison, Wis, USA), 2 µM of each primer, and 40 ng of genomic DNA that was used as a template. All PCR mixes were overlaid with mineral oil and reactions were carried out in a Perkin Elmer DNA Thermal Cycler 480, programmed for 4 min at 95 °C for an initial denaturation, and 36 cycles of 1 min at 94 °C (DNA denaturation), 1 min at 50 °C (primer annealing) and 1.5 min at 72 °C (primer extension), followed by a final 7-min extension at 72 °C. Denaturing polyacrylamide-gel electrophoresis (PAGE) and a sequencing gel apparatus (420 × 330 × 0.4 mm) were used to separate amplified fragments. This system allowed separation of heterogeneous RGA fragments and detection of individual bands, as described elsewhere (Chen et al. 1998). After electrophoresis, the gel, fixed to the Bind-Silane surface of one glass plate, was silver-stained following the manufacturer's protocol (Promega). The gel was air-dried overnight at room temperature and stored in darkness for scoring and scanning.

Table 1 Oligonucleotide primers used for the amplification of resistance gene analogs (RGAs). The design bases and sources of the primers are shown

Group	Primers	Sequences (5′–3′)[a]	Design basis	References
LRR	RLRR for. RLRR rev.	CGCAACCACTAGAGTAAC ACACTGGTCCATGAGGTT	LRR domain in the *RPS2* gene conferring resistance to *Pseudomonas syringae* in *Arabidopsis*	Chen et al. 1998 (barley, wheat and rice)
	XLRR for. XLRR rev.	CCGTTGGACAGGAAGGAG CCCATAGACCGGACTGTT	LRR domain of the rice *Xa21* gene conferring resistance to *Xanthomonas campestris* pv *oryzae*	
	CLRR for. CLRR rev.	TTTTCGTGTTCAACGACG TAACGTCTATCGACTTCT	LRR domain of the tomato *Cf-9* geneconferring resistance to *Cladosporium fulvum*	
NBS	ANo. 2 ANo. 3	TATAGCGGCCGCIARIGCIARIGGIARNCC ATATGCGGCCGCGGIGGIGTIGGIAARACNAC	Conserved P-loop and hydrophobic NBS regions of the *N* and *RPS2* genes from tobacco and *Arabidopsis* respectively	Speulman et al. 1998 (*Arabidopsis*)
	S1 AS1 S2 AS3	GGTGGGGTTGGGAAGACAACG CAACGCTAGTGGCAATCC GGIGGIGTIGGIAAIACIAC IAGIGCIAGIGGIAGICC	Hydrophobic domain and P-loop of conserved NBS from the *Arabidopsis N* and *RPS2* genes and the flax *L6* gene conferring resistance to rust	Leister et al. 1996 (potato) Mago et al. 1999 (rice)
PtoKin	Ptokin1 Ptokin2 Ptokin3 Ptokin4	GCATTGGAACAAGGTGAA AGGGGGACCACCACGTAG TAGTTCGGACGTTTACAT AGTGTCTTGTAGGGTATC	Serine/threonine protein kinase domain of the tomato *Pto* gene conferring resistance to the bacterial pathogen *Pseudomonas syringae* pv *tomato*	Chen et al. 1998 (barley, wheat and rice) Leung, H. (personal communication)[b]

[a] Code for mixed bases: I = Inosine; N = A/G/C/T, R =A/G
[b] The International Rice Research Institute (IRRI), The Philippines

To determine the size of polymorphic RGA fragments, DNA bands were isolated from the dried polyacrylamide gel and directly re-amplified, using a needle scratching and PCR re-amplification method (Stumm 1997). The re-amplified products and DNA size markers (1 kb, 100 bp, 50 bp) were electrophoresed on a 1.0% agarose gel, stained with ethidium bromide and photographed.

Map construction

The BC_1 plants were genotyped for 141 RFLP and 23 RGA markers and a genetic linkage map was constructed using the computer program MAPMAKER v. 3.0 (Lander et al. 1987). The proportion of the recurrent parent (*L. esculentum*) genome in each BC_1 plant and its distribution in the BC_1 population were also determined, using the computer program QGENE v. 3.04 (Nelson 1997). The linkage map was used for QTL identification in the BC_1, BC_1S_1-1999 and BC_1S_1-2000 populations.

QTL identification

Two analytical approaches were employed to identify and validate putative QTLs and estimate their phenotypic effects. First, simple interval mapping (SIM) analysis, using the computer program MAPMAKER/QTL v. 1.1 (Lincoln et al. 1992), was employed to identify marker intervals on the tomato chromosomes that contained QTLs. To identify the appropriate threshold LOD score [$\log_{10}$ of odds ratio (Lander and Botstein 1989)] for declaring a QTL, given the population size and the number of markers used, a 1,000 × permutation test was first conducted (Churchill and Doerge 1994) using the QGENE computer program. The permutation test resulted in LOD threshold values of 2.4 for the BC_1 and 2.3 for the BC_1S_1-1999 and BC_1S_1-2000 populations. The LOD scores obtained from SIM analysis were used to construct QTL likelihood plots (Lander and Botstein 1989; Paterson et al. 1991) of detected QTLs on different chromosomes, using Microsoft Excel version 5.0 for Macintosh. The MAPMAKER/QTL program was also used to obtain estimates of the percentage of the total phenotypic variation explained (PVE) by each QTL. Phenotypic effects of each QTL interval (in AUDPC units) was also determined by MAPMAKER/QTL. Furthermore, the bi-locus and multi-locus models from the MAPMAKER/QTL program were used to estimate the PVEs for various combinations of QTLs.

The second approach used for QTL identification was the composite interval mapping (CIM) analysis (Zeng 1994) employing model 6 of the QTL Cartographer computer program (Basten et al. 1999). This method is a multiple regression procedure adjusting for background effects of markers (co-factors) other than those in the interval being tested. The program's 'SRMAPQTL' feature, a forward regression with backward elimination (FB), was utilized to choose co-factors before performing QTL detection. Only markers (co-factors) with a significance level of $P < 0.1$ were considered. Three co-factors (QTL-linked markers with the highest F values) were used for the CIM analysis, using the feature 'ZMAPQTL'. The walking speed of 2 cM was chosen, and the window size around the test interval, the region not considered as a background co-factor, was set to 10 cM. A 1000 × permutation test was performed to estimate appropriate significant threshold LOD scores for CIM. A LOD threshold level of 2.4 was used for both BC_1 and BC_1S_1 populations.

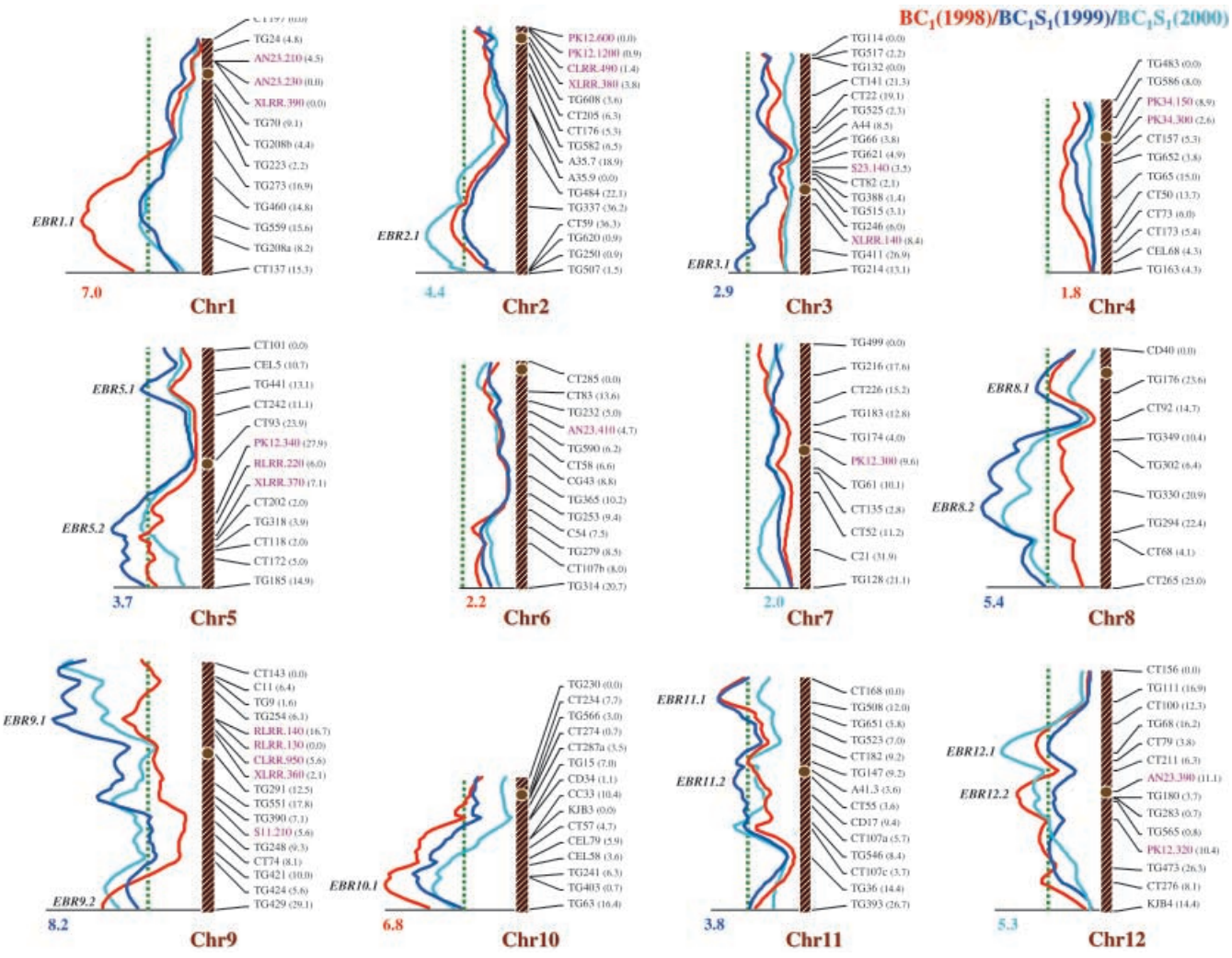

Fig. 1 A linkage map of the 12 tomato chromosomes constructed based on a BC_1 population of a cross between *L. esculentum* breeding line NC84173 (EB susceptible) and *L. hirsutum* accession PI126445 (EB resistant). The map includes 141 RFLP markers (shown in *black font*) and 23 resistance gene analogs (RGAs; *pink font*). The names of the markers and map distances between them are shown at the right of the chromosomes. The LOD (log 10 of the odd ratio) plots at the left of the chromosomes indicate the most likely positions of QTLs for EB resistance identified in the BC_1 (*red curves*), BC_1S_1-1999 (*dark blue curves*) and BC_1S_1-2000 (*light blue curves*) populations. The LOD plots were derived based on single interval mapping (SIM) using the QGene computer program. The height of each LOD curve indicates the strength of the evidence for the presence of a QTL at each location. The *dotted green* vertical lines indicate a LOD value of 2.4, a threshold value that the LOD score must cross to allow the presence of a QTL to be inferred. The maximum-likelihood position of the QTL(s) is the highest point on the curve, which is shown at the left of the chromosomes together with the name(s) of the QTL(s). The highest LOD score obtained for each chromosome is also shown on the Y axis

Result and discussion

Genetic linkage map and genome distribution in the BC_1 population

A genetic linkage map was constructed with 141 RFLP and 23 RGA markers, spanning approximately 1,409 cM of the tomato genome with an average distance of 8.6 cM between markers (Fig. 1). The length of the map and the order of the markers were in agreement with the high-density RFLP map of tomato, which was previously constructed based on a *L. esculentum* × *Lycopersicon pennellii* F_2 population (Pillen et al. 1996).

Genome composition of the 145 BC_1 plants ranged from 64.3% to 99.4% from the E parent, with an average of 80.8% (Fig. 2). This average was rather larger than the expected 75% for a BC_1 population, but was not unexpected because of: (1) the use of an interspecific cross, and (2) the selections made against self-incompatibility, indeterminant growth habit and late maturity, all three traits contributed from the H genome. Skewed segregation has been reported in most interspecific crosses of tomato, with the extent of skewness often being greater in wider crosses (e.g.), *L. esculentum* × *L. pennellii* and

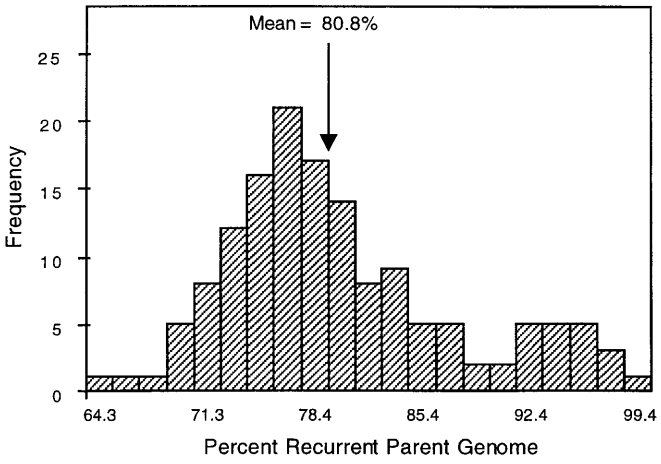

Fig. 2 Distribution of the percent recurrent parent (NC84173) genome in the NC84173 × PI126445 BC_1 population

L. esculentum × L. hirsutum) than crosses between closely related species (e.g.), *L. esculentum* × *L. pimpinellifolium* and *L. esculentum* × *Lycopersicon cheesmanii*), and generally higher in filial (average 70%) than in backcross populations (average 40%) (Bernacchi and Tanksley 1997). Furthermore, skewed segregation in interspecific crosses of tomato has been attributed to various causes, including SI, unilateral incongruity, gametophytic selection, and viability selection of segregating plants (Lewis and Crowe 1958; Trognitz and Schmiediche 1993; Foolad 1996). In the present study, the extent of skewness was rather large (62%), although only markers on chromosomes 1, 5 and 6 were severely skewed. Most likely, selections against SI, indeterminant growth habit and late maturity contributed to a high level of skewness in this population. Such selections, however, were necessary in order to identify QTLs with true effects on EB resistance, as described below (section on QTL identification). The highest level of skewness was observed for markers on chromosome 1, in particular for markers on the short arm of this chromosome, where the *S* locus (for SI) has been mapped (Tanksley and Loaiza-Figueroa 1985; Pillen et al. 1996). Three markers at the telomeric end of the long arm of this chromosome exhibited normal segregation. Similar skewed segregation for markers on chromosome 1 was previously reported in other interspecific crosses of tomato where marker-assisted selection (MAS) was performed for self-compatibility before conducting QTL mapping (Fulton et al. 1997b; Bernacchi et al. 1998). The second highest level of skewed segregation was observed for markers on chromosome 6 on which the *sp* (self-pruning) locus is located (Paterson et al. 1988; Grandillo and Tanksley 1996a; Fulton et al. 1997b). Similarly, skewed segregation for markers on this chromosome was previously reported in other interspecific crosses of tomato where MAS was performed for the determinant plant type before conducting QTL mapping (Grandillo and Tanksley 1996a; Bernacchi and Tanksley 1997; Pnueli et al. 1998). In tomato, self-incompatibility and indeterminant growth habit are undesirable characteristics for genetics and breeding studies (including QTL mapping) as well as commercial production under field conditions (Stevens and Rick 1986; Bernacchi and Tanksley 1997; Fulton et al. 1997b).

Response of parental, F_1 and backcross generations to EB disease

The final percent defoliation and/or AUDPC values for the parental lines and F_1, BC_1 and BC_1S_1 progeny are presented in Table 2. Because of the presence of a significant correlation ($r \geq 0.97$, $P < 0.01$) between the final percent defoliation and AUDPC values in both BC_1 and BC_1S_1-1999 populations and because QTL results were almost identical based on both measures of resistance, only AUDPC results are discussed in detail (except for the BC_1S_1-2000 population for which AUDPC values were not available). The two parental lines exhibited extreme responses to EB infection, with NC84173 being highly susceptible and PI126445 being highly resistant; the mean AUDPC value for NC84173 was approximately 37-times that for PI126445 (Table 2). For each parent,

Table 2 Early blight (EB) disease severity (final percent defoliation, ±SE) and the area under the disease progress curve (AUDPC, ± SE) for the parental lines and BC_1 and BC_1S_1 progeny of a cross between *L. esculentum* breeding line NC84173 (EB susceptible) and *L. hirsutum* accession PI126445(EB resistant)

Generation	Growth habit[a]	*n*[b]	Final percent defoliation		AUDPC	
			Average	Range	Average	Range
P_1 (NC84173) (1999)	D	150	99.7±0.6	99.0–100.0	2,154±133	2,001–2,242
P_2 (PI126445) (1999)	I	150	5.0±0.0	5.0–5.0	58±0	58–58
F_1 ($P_1 \times P_2$) (1999)	I	30	8.3±1.5	7.0–10.0	153±18	138–172
BC_1 (1998)	D	146	50.1±23.4	15.0–100.0	814±433	228–2,030
BC_1S_1 (1999)	D	146	59.2±18.7	25.7–100.0	1,153±393	460–2,208
BC_1S_1 (2000)	D	146	69.5±17.8	25.0–100.0	NA[c]	NA

[a] D = Determinant, I = Indeterminant

[b] *n* = Total number of plants (for the P_1, P_2 and BC_1) or families (for the BC_1S_1) evaluated for EB resistance. For the BC_1S_1-1999 population 30 plants of each family and for BC_1S_1-2000 population 20 plants of each family were evaluated

[c] Not available

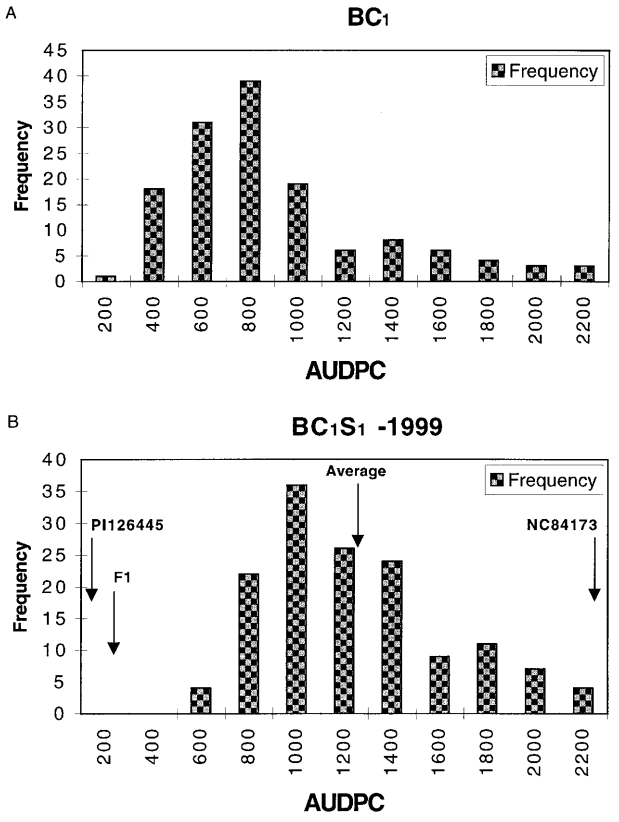

Fig. 3 Frequency distributions of AUDPC values for the BC_1 **(A)** and BC_1S_1-1999 **(B)** populations

there was little or no variation within or between plots for EB symptoms (Table 2). Plants of the F_1 generation were indeterminate and highly vigorous in growth habit and were nearly free of EB disease, similar to the resistant parent. Also, there was little variation within or between plots for EB symptoms in the F_1 generation (Table 2).

The mean AUDPC and/or final percent defoliation values for the BC_1, BC_1S_1-1999 and BC_1S_1-2000 populations were intermediate between the two parents (Table 2). ANOVA indicated the presence of significant ($P < 0.05$) differences among the BC_1 plants in EB resistance, with the AUDPC ranging from 228 to 2,030 (Table 2) and exhibiting a continuous distribution (Fig. 3A), typical of quantitative traits. Similarly, there were significant differences among the BC_1S_1-1999 and BC_1S_1-2000 families, with the AUDPC ranging from 460 to 2,208 (for the BC_1S_1-1999) and the final percent defoliation ranging from about 25% to 100% (for both BC_1S_1-1999 and BC_1S_1-2000) (Table 2) and exhibiting continuous distributions (Fig. 3B; shown only for the BC_1S_1-1999 population). However, generally more EB was observed in the BC_1S_1-1999 than in the BC_1 population (Fig. 3). This could be due to various reasons including: (1) partial dominance of resistance and a higher level of homozygosity in the BC_1S_1 (average 75%) than in the BC_1 generation (50%), and (2) a higher level of EB epiphytotic in 1999. Similarly, a higher level of EB (final percent defoliation) was recorded for the BC_1S_1-2000 population compared to that in the BC_1S_1-1999 population (Table 2). Most likely this was due to a later date of final disease evaluation in the BC_1S_1-2000 population (September 28) than in the BC_1S_1-1999 population (September 17). However, the distribution of the final percent defoliation in the BC_1S_1-2000 was highly similar to that for the BC_1S_1-1999 (data not shown). As expected, all of the BC_1S_1 families were self-compatible and determinant in growth habit, and thus suitable for screening for EB resistance and mapping QTLs as well as for breeding research. No transgressive segregant for resistance was observed in either the BC_1 or the BC_1S_1 generation, and no BC_1 plant or BC_1S_1 family was found with a resistance similar to that of the resistant parent, PI126445.

Across the BC_1S_1-1999 families, a small, but significant, negative correlation ($r = -0.26$, $P < 0.01$) was observed between disease resistance (AUDPC values) and earliness-in-maturity. This correlation indicates that the recorded resistance was somewhat affected by plant maturity and that, despite the selections made against late maturity in the BC_1 generation, there was still variation in maturity in the BC_1S_1 population. The negative relationship between early maturity and the occurrence of EB must be considered when conducting QTL mapping or selecting plants for EB resistance using progeny derived from PI126445. Perhaps, for practical purposes, plants with late maturity should be eliminated in early backcross generations. The use of large-size populations may facilitate the identification of recombinants with EB resistance and acceptable maturity. In the present study, we found a few BC_1S_1 families with considerable resistance to EB and yet with mid-season maturity. These identified families should be useful for the development of commercially acceptable EB-resistant tomato lines using MAS or traditional breeding approaches.

In general, there were great similarities between EB resistance/susceptibility of the BC_1 plants in 1998 and the corresponding BC_1S_1 families in 1999 and 2000, as evident by significant correlations ($r = 0.69$–0.70, $P < 0.01$) between the AUDPC and/or %defoliation values of the two generations. Such correlation coefficients are equivalent to parent-offspring regression estimates of heritability computed based on standard-unit data (Frey and Horner 1957; Dudley and Moll 1969; Falconer 1989).

QTL identification and validation

Effects of selections made in the BC_1 population on identification of QTLs for EB resistance

EB resistance in tomato is negatively correlated with earliness and positively correlated with plant physiological maturity and fruit load (Barratt and Richards 1944; Nash and Gardner 1988b). Late maturing and low yielding plants (including self-incompatible plants, which may not produce any fruit) appear resistant, while they

may not possess genetic resistance. Similarly, indeterminant plants may outgrow the disease and emerge as resistant, while they may not have genes for EB resistance. Thus, plant evaluation for EB resistance in a population that is segregating for self-incompatibility (SI), growth habit and/or late maturity would be greatly confounded by the effects of such factors on plant disease response. A consequence of such practice in mapping populations would be the misscoring plants in their disease response and thus the detection of QTLs that do not actually represent genes for EB resistance, but genomic regions associated with SI, indeterminant growth habit and/or late maturity. Obviously, such QTLs would be useless for MAS and breeding for EB resistance. Furthermore, SI and indeterminant growth habit are unacceptable characteristics for commercial tomato production in the field (Stevens and Rick 1986; Fulton et al. 1997b). When using wild tomato germplasms (which are mostly indeterminant and self-incompatible) as genetic resources for breeding purposes or QTL mapping, it is essential that in early backcross generations plants with SI or indeterminant growth habit (both single-gene traits) are eliminated. This is routinely done in many tomato mapping and breeding research programs, using either MAS (Tanksley et al. 1996; Fulton et al. 1997a; Bernacchi et al. 1998) or phenotypic screening (R.G. Gardner, N.C. State University; personal communication). Similarly, late maturity is an undesirable characteristic, in particular for tomato production in temperate regions with short growing seasons.

In the present study, to avoid such confounding effects, only BC_1 plants that were self-compatible and determinant with early to mid-season maturity were used for trait evaluation and QTL identification. This process allowed the identification of QTLs with true effects on EB resistance and which would be more reliable for MAS and breeding. However, a potential drawback of the selections made in the BC_1 population would be the inability to scan for QTLs in chromosomal regions where genes for the selected traits (SI, growth habit and lateness-in-maturity) were located. For example, in tomato, the *S* locus (for SI) is located on the short arm of chromosome 1 near the centromere and closely linked to RFLP markers CT197 and TG24 (Tanksley and Loaiza-Figueroa 1985; Pillen et al. 1996) and the *sp* locus (for self-pruning; conferring a determinant growth habit) is located on chromosome 6 near RFLP marker TG279 (Paterson et al. 1988; Grandillo and Tanksley 1996a; Fulton et al. 1997b). Any putative QTLs in these regions or in regions with significant effects on lateness-in-maturity could not be detected in the present study. However, this drawback is much less critical than problems associated with the presence of SI, an indeterminant growth habit and late-maturity in the mapping populations. It should also be noted that, for practical reasons, in populations segregating for SI, an indeterminant growth habit and late-maturity, it would be extremely difficult to identify EB resistance QTLs closely linked to genes controlling these traits. This is because of the significant confounding effects of these factors on EB resistance. Furthermore, there is also the possibility that no QTL for EB resistance exists in such regions, and there is no published information as to the existence of such genetic linkages.

QTL identification in the BC_1 population

The SIM analysis identified nine significant QTLs (LOD $\geq$ 2.4, $P \leq 0.001$) on chromosomes 1, 2, 5, 9, 10, 11 and 12 for EB resistance in the BC_1 population, with individual effects ranging from 8.4% (*EBR5.2*) to 21.9% (*EBR1.1*) (Table 3; Fig. 1). Multi-locus analysis indicated that the combined effects of these QTLs accounted for 56.9% of the total phenotypic variation, which was significantly less than the sum of individual effects of the nine QTLs (133.3%). This difference could be due to less-than-additive epistatic interactions among QTLs (Eshed and Zamir 1996) or to the possibility that some QTLs affect EB resistance through the same developmental pathways and are not independent of each other. In addition to the nine significant QTLs, SIM analysis detected two minor QTLs (LOD = 2.3, $P = 0.0013$, PVE = 7.4%) on chromosomes 8 (*EBR8.1*) and 11 (*EBR11.2*) in the BC_1 population; these two QTLs, however, were detected as major QTLs (LOD = 3.0, $P \leq 0.0007$, PVE $\geq$ 9.9%) in the BC_1S_1 population using SIM analysis (described below). All QTLs had the positive alleles (conferring resistance) from the disease resistant parent.

The CIM analysis, using Model 6 of the QTL Cartographer program with three co-factors (QTL-linked markers with highest *F* values), confirmed 6 of the 11 QTLs as being independent of each other and having significant effects (LOD $\geq$ 2.4). These QTLs were located on chromosomes 1, 5, 9 (two QTLs), 10 and 11, with individual effects ranging from 7.1% (*EBR11.2*) to 15.1% (*EBR1.1*) and combined effects of 52.6% of the total phenotypic variation (Table 3). The use of a larger number of co-factors did not significantly affect the detection and individual effects of the QTLs. Thus, the identified QTLs should be real and useful for MAS (see below).

QTL identification in the BC_1S_1-1999 population

The SIM analysis identified 13 significant QTLs (LOD $\geq$ 2.4, $P \leq 0.001$) on chromosomes 1, 2, 3, 5, 8, 9, 10, 11 and 12 for EB resistance in the BC_1S_1-1999 population, with individual effects ranging from 7.9% (*EBR5.1*) to 25.9% (*EBR9.1*) (Table 3, Fig. 1). Multi-locus analysis indicated that the combined effects of these QTLs accounted for 57.8% of the total phenotypic variation, significantly less that the sum of individual effects of all QTLs (175.5%). It is interesting that even with the detection of 13 QTLs, only slightly over half of the phenotypic variation could be explained by the combined effects of the QTLs. The rest of the variation was most likely due to the effect of QTLs which remained undetected in

Table 3 QTLs detected for early blight (EB) resistance based on simple interval mapping (SIM) and composite interval mapping (CIM) in BC_1 and BC_1S_1 populations of an interspecific cross between *L. esculentum* (NC84173; EB susceptible) and *L. hirsutum* (PI126445; EB resistant). LOD = log-likelihood; PVE = percent phenotypic variation explained; *E* = *L. esculentum* allele; *H* = *L. hirsutum* allele; Phenotypic effect = difference between *E*/*H* and *E*/*E* in AUDPC

QTLs	Interval	Chromosome	SIM			CIM	
			LOD	PVE	Phenotypic effect %	LOD	PVE %
BC_1							
EBR1.1	TG559–TG208a	1	7.0	21.9	–409.9	5.9	15.1
EBR2.1	TG337–CT59	2	2.9	15.3	–343.0	2.2ns[a]	7.3
EBR5.2	XLRR.370–CT202	5	2.6	8.4	–295.9	2.4	7.3
EBR8.1	CD40–TG176	8	2.3ns	7.4	–246.8	1.3ns	3.5
EBR9.1	RLRR.130–CLRR.950	9	4.2	14.9	–336.1	2.8	7.5
EBR9.2	TG424–TG429	9	5.1	16.2	–352.8	3.8	10.1
EBR10.1	TG241–TG403	10	6.8	20.2	–397.3	5.0	10.8
EBR11.1	CT168–TG508	11	3.8	13.2	–332.2	2.1ns	5.2
EBR11.2	TG147–A41.3	11	2.3ns	7.4	–269.5	3.2	7.1
EBR12.1	CT100–TG68	12	3.1	10.3	–298.6	1.2ns	3.0
EBR12.2	AN23.390–TG180	12	4.1	12.9	–335.5	1.8ns	4.3
BC_1S_1-1999							
EBR1.1	TG559–TG208a	1	3.6	11.9	–273.8	3.5	7.5
EBR2.1	TG337–CT59	2	2.8	15.9	–317.0	1.8ns	5.4
EBR3.1	TG411–TG214	3	2.9	9.1	–272.7	2.2ns	7.0
EBR5.1	TG441–CT242	5	2.6	7.9	–234.3	0.3ns	0.5
EBR5.2	XLRR.370–CT202	5	3.7	11.3	–310.3	3.6	9.2
EBR8.1	CD40–TG176	8	3.0	10.3	–262.6	3.7	9.0
EBR8.2	TG330–TG294	8	5.4	21.0	–363.5	5.2	14.3
EBR9.1	RLRR.130–CLRR.950	9	8.2	25.9	–402.7	8.1	21.9
EBR9.2	TG424–TG429	9	3.7	16.2	–307.1	1.8ns	4.2
EBR10.1	TG241–TG403	10	5.6	16.3	–322.3	4.1	10.1
EBR11.1	TG508–TG651	11	3.8	11.5	–279.0	1.9ns	4.2
EBR11.2	CT55–CD17	11	3.0	9.9	–270.9	2.0ns	4.5
EBR12.1	CT100–TG68	12	2.5	8.3	–241.6	1.7ns	4.0

[a] ns: not significant

this study and/or epistatic interactions (see below). Similar to that in the BC_1 population, all QTLs had the positive alleles contributed from the disease-resistant parent. All but one QTL (*EBR12.2*) that were identified in the BC_1 population were also detected in the BC_1S_1-1999 population (Table 3); however, *EBR12.2* was also detected as a minor QTL in the BC_1S_1-1999 and as a major QTL in the BC_1S_1-2000 population (Fig. 3).

The CIM analysis, using Model 6 of the QTL Cartographer program with three co-factors, confirmed 6 of the 13 QTLs, located on chromosomes 1, 5, 8 (two QTLs), 9 and 10, as being independent of each other and having significant individual effects ranging from 7.5% (*EBR1.1*) to 21.9% (*EBR9.1*). Together these six QTLs could account for 53.1% of the total phenotypic variation (Table 3). Results of the CIM analyses were similar in the BC_1 and BC_1S_1 populations (described below).

QTL identification in the BC_1S_1-2000 population

Results of QTL mapping using SIM and CIM analyses in the BC_1S_1-2000 population (using the final percent defoliation) were highly similar to those for the BC_1 and BC_1S_1-1999 populations. Thus, the QTL results for BC_1S_1-2000 are not presented in Table 3 and are not discussed in much detail. However, the position of QTLs identified in the BC_1S_1-2000 population is shown in Fig. 1.

Analysis of QTLs identified on the same chromosomes

On each of chromosomes 5, 8, 9, 11 and 12, more than one QTL-likelihood peak was detected based on SIM analysis (Fig. 1, Table 3). It is important to determine whether different QTL peaks on a chromosome represent different QTLs with an independent (or related) function or if there is only one QTL on each chromosome. Two approaches were taken to distinguish between these possibilities. First, using the CIM analysis, by considering one putative QTL as a co-factor, we examined the effects (%PVE) of the other QTL(s). In the second approach, using bi-locus analysis of the MAPMAKER/QTL program, we examined the combined effects of the putative QTLs on each chromosome and compared that with their sum of individual effects. Results of the two approaches were highly similar. For example, for the three comparisons made in the BC_1 generation for QTLs on each of chromosomes 9, 11 and 12, both analyses indicated the presence of two QTLs on chromosome 9 (*EBR9.1* and *EBR9.2*) but only one QTL on each of chromosome 11

Table 4 Individual and combined effects [percent variation explained (%PVE)] of QTLs identified on the same chromosomes. "Composite analysis" displays the %PVE of the two QTLs on the samechromosome when one was used as the background co-factor. "Combination of QTLs" displays the combined effects of the two QTLs as determined by bi-locus analysis and the sum of the individual effects of the two QTLs

QTL	Chrom.	Composite analysis		Combinations of QTLs	
		QTL 1	QTL 2	Bi-locus model	Sum of 2 QTLs
BC_1					
EBR 9.1 (QTL 1)[a]	9	14.8	12.5		
EBR 9.2 (QTL 2)[a]	9	10.3	16.1		
EBR 9.1/EBR 9.2	9, 9			27.3	31.3
EBR 11.1 (QTL 1)[a]	11	13.2	0.2ns		
EBR 11.2 (QTL 2)[a]	11	4.4ns[b]	7.3		
EBR 11.1/EBR 11.2	11, 11			13.6	20.6
EBR 12.1 (QTL 1)[a]	12	11.9	4.4ns		
EBR 12.2 (QTL 2)[a]	12	0.9ns	12.8		
EBR 12.1/EBR 12.2	12, 12			14.9	23.2
BC_1S_1-1999					
EBR 5.1 (QTL 1)[a]	5	7.8	6.0ns		
EBR 5.2 (QTL 2)[a]	5	2.5ns	11.2		
EBR 5.1/EBR 5.2	5, 5			13.9	19.2
EBR 8.1 (QTL 1)[a]	8	10.3	14.7		
EBR 8.2 (QTL 2)[a]	8	10.3	14.7		
EBR 8.1/EBR 8.2	8, 8			26.1	31.3
EBR 9.1 (QTL 1)[a]	9	24.3	15.4		
EBR 9.2 (QTL 2)[a]	9	21.1	15.0		
EBR 9.1/EBR 9.2	9, 9			31.5	40.9
EBR 11.1 (QTL 1)[a]	11	11.4	1.6ns		
EBR 11.2 (QTL 2)[a]	11	4.0ns	9.9		
EBR 11.1/EBR 11.2	11, 11			13.1	21.4

[a] QTL used as the background co-factor
[b] ns = not significant as determined by LOD score (LOD < 2.4)

(possibly *EBR11.1*) and 12 (possibly *EBR12.2*). For the two QTLs on chromosome 9, the bi-locus analysis indicated that their combined effects (PVE = 27.3%) were similar to the sum of their individual effects (PVE = 31.1%), and the CIM analysis indicated the presence of two QTLs on this chromosome (i.e., using one QTL as a co-factor did not significantly change the PVE of the other) (Table 4). In contrast, for the two putative QTLs identified on chromosomes 11 and 12 both analyses indicated the presence of colinearity effects, suggesting the presence of only one QTL (or two functionally related QTLs) on each chromosome (Table 4). Similarly, the two analyses indicated the presence of two QTLs on each of chromosomes 8 and 9 and the presence of only one QTL on each of chromosomes 5 and 11 in the BC_1S_1-1999 population (Table 4). Furthermore, as described in the previous section, the CIM analysis detected two QTLs on chromosome 9 in the BC_1 and two QTLs on chromosome 8 in the BC_1S_1-1999 population. Thus, the overall results suggest the presence of two separate QTLs on each of chromosomes 8 (*EBR8.1* and *EBR8.2*) and 9 (*EBR9.1* and *EBR9.2*), and only one QTL on each of chromosomes 5 (possibly *EBR5.2*), 11 (possibly *EBR11.1*) and 12 (possibly *EBR12.2*). This information should be useful when planning for MAS and breeding for EB resistance using PI126445 as a gene resource (see below).

Epistatic interactions

Pair-wise epistatic interactions between all markers (a total of 13,366 possible interactions) were examined using QGENE in both BC_1 and BC_1S_1-1999 populations. Totals of 299 (2.23% of the total) and 445 (3.33%) significant ($P \leq 0.002$, $F \geq 10.00$) pair-wise epistatic interactions were identified in the BC_1 and BC_1S_1-1999 populations, respectively. The identified interactions were of three types. (1) Interactions between QTL-linked markers (i.e., between putative QTLs), of which 26 (0.19%) and 64 (0.47%) were identified in the BC_1 and BC_1S_1-1999 populations, respectively. (2) Interactions between QTL-linked and QTL-unlinked markers, of which 55 (0.41%) and 123 (0.92%) were identified in the BC_1 and BC_1S_1-1999 populations, respectively. (3) Interactions between QTL-unlinked markers, for which 218 (1.63%) and 258 (1.93%) were identified in BC_1 and BC_1S_1-1999 populations, respectively. The percentages of these interactions were smaller than the percentage expected (5%) to occur by chance (Paterson et al. 1991; deVicente and Tanksley 1993), indicating that all or some of these epistatic interactions might be results of chance events. However, the observation that most of the identified epistatic interactions were between QTL-unlinked markers suggests the potential presence of interactive QTLs whose individual effects could not be detected by the

Table 5 Best combinations of QTLs for early blight resistance (EBR) in BC_1 and BC_1S_1 populations of a cross between *L. esculentum* breeding line NC84173 and *L. hirsutum* accession PI126445. LOD = log-likelihood for combined QTLs; PVE = percentage phenotypic variation explained by each combination of QTLs

Combined QTLs	Chromosome	BC_1		BC_1S_1-1999	
		LOD	PVE	LOD	PVE
EBR9.1/EBR1.1	9, 1	10.4	31.9	11.0	32.5
EBR9.1/EBR1.1/EBR10.1	9, 1, 10	14.0	39.0	13.5	37.5
BR9.1/EBR1.1/EBR10.1/EBR8.2	9, 1, 10, 8	14.5	40.1	16.9	47.1
BR9.1/EBR1.1/EBR10.1/EBR8.2/EBR5.2	9, 1, 10, 8, 5	17.1	45.0	20.8	53.1
BR9.1/EBR1.1/EBR10.1/EBR8.2/EBR5.2/EBR2.1	9, 1, 10, 8, 5, 2	19.1	50.3	22.6	56.4

current QTL mapping techniques. The presence of such epistatic interactions is a likely cause for the large amount of unexplained phenotypic variation by the identified QTLs (Li et al. 1997; Zhu et al. 1999).

In a subsequent analysis, the identified epistatic interactions were grouped into pair-wise epistatic interactions between regions (each region containing one or more marker locus), which again were of three types: interactions between QTL-linked regions, of which 5 were identified in BC_1 and 11 in BC_1S_1, interactions between QTL-linked and QTL-unlinked regions, of which 29 were identified in BC_1 and 39 in BC_1S_1, and interactions between QTL-unlinked regions, of which 13 were identified in BC_1 and 16 in BC_1S_1. However, no single interaction was identified between any two QTL-unlinked regions or between a QTL-linked and a QTL-unlinked region that could contribute to disease resistance more than any combination of two QTL-linked regions. Thus, only interactions between QTL-linked regions were inspected further.

Of the 78 possible digenic epistatic interactions between the 13 putative QTLs (using the nearest marker loci for the analyses), only 5 in the BC_1 and 11 in the BC_1S_1 populations were significant ($P \leq 0.002$, $F \geq 10.00$) (data not shown). Of these, however, only one interaction, between *EBR1.1* and *EBR10.1*, was consistently significant in both BC_1 and BC_1S_1 generations. In almost every case, the significance of the interaction resulted when the combined effects of the two QTLs were less than the sum of their individual effects. This indicates that in such cases effects of the two putative QTLs were not independent of each other, which may be a reason for the less-than-additive effects of most QTLs. The exact nature of such epistatic interactions could not be determined in the present study. Development of near-isogenic lines for single and multiple QTLs may facilitate precise determination of the presence and nature of epistatic interactions. However, most previous QTL mapping experiments have revealed very limited or no interactions among QTLs (Paterson et al. 1988, 1990; Stuber et al. 1992; deVicente and Tanksley 1993; Cocherham and Zeng 1996; Grandillo and Tanksley 1996b) whereas a few others have suggested the importance of epistatic interactions (Doebley et al. 1995; Lark et al. 1995; Li et al. 1997). For breeding purposes using MAS, however, QTLs that do not require epistatic interactions are more desirable.

Comparison of QTLs across populations and determination of best QTL combinations for MAS

QTL results were highly similar across populations and years, suggesting the stability of the identified QTLs and their potential utility for MAS and transfer of resistance from PI126445 to the cultivated tomato. The consistent results obtained across populations (see Fig. 1) is also indicative of accurate trait evaluation in different years. However, the total number of identified QTLs was rather large and it might not be technically feasible to transfer all of the QTLs via MAS. Generally, as the number of genomic regions to be transferred increases, the utility of MAS becomes questionable because larger and larger populations are needed to identify favorable QTL combinations. Using both MAPMAKER/QTL and QTL Cartographer programs, we attempted to identify smaller combinations of QTLs which could account for a significant portion of the total phenotypic variation. The selection of each combination was based on several criteria, including the expression (detection) of QTLs in different generations and years, the independence of QTL effects, and the magnitude of QTL effects. Furthermore, greater emphasis was placed on QTLs detected in the BC_1S_1-1999 population, where trait evaluation was based on replicated family performance rather than individual plant performance (as in the BC_1 population). We examined different combinations of QTLs and selected the best combinations of 2–6 QTLs (Table 5). Results of the analyses indicated that a combination of five or six of the selected QTLs could account for >50% of the total phenotypic variation in the BC_1S_1-1999 population (Table 5). The analyses also indicated comparable PVEs for each set of selected QTLs in the BC_1 and BC_1S_1-1999 populations (Table 5). Further inspections indicated that the six selected QTLs were more or less independent of each other with additive effects, and thus should be the most useful QTLs for MAS and breeding using PI126445 as a gene resource. Simultaneous introgression of five QTLs into the cultivated tomato by MAS is feasible, providing opportunities to rapidly develop tomato cultivars with enhanced EB resistance.

The overall QTL analyses indicated that a majority of the variation (>50%) for EB resistance in the BC_1 and BC_1S_1 generations could be explained by combined effects of five QTLs (Table 5) with their individual effects

ranging from about 10% to 25% of the total phenotypic variation (Table 3). These results are consistent with the hypothesis that quantitative traits can be controlled by the effects of a few major QTLs which act in concert with a number of smaller-effect QTLs (Lande and Thompson 1990). This hypothesis has also been supported by several previous investigations which indicated the presence of several major QTLs for different quantitative traits (Paterson et al. 1991; deVicente and Tanksley 1993; Foolad et al. 1998). However, it still needs to be determined whether transfer of such a small number of QTLs would make significant improvement in the trait(s) of interest.

Relationship between genomic locations of RGAs and QTLs

Linkage analysis indicated that the 23 polymorphic RGA markers were mapped to 9 of the 12 chromosomes (Fig. 1). The results supported the previous suggestion that RGA bands resolved in denaturing polyacrylamide gels represent individual DNA fragments and can be used as genetic markers for genome mapping (Chen et al. 1998). On chromosomes 1, 2, 5 and 9, RGA loci were clustered, similar to those previously reported for resistance genes (R genes) and RGAs in various plant species (Kanazin et al. 1996; Yu et al. 1996; DeJong et al. 1997; Aarts et al. 1998; Ashfield et al. 1998; Simons et al. 1998; Speulman et al. 1998; Thomas et al. 1998). RGA markers were assessed for their positional association with QTLs identified for EB resistance. Among the 23 RGA loci, the positions of ten (43.5%) coincided with positions of QTLs identified on chromosomes 5, 9 and 12 (Fig. 1). Whether these RGAs are a part of genes (QTLs) conferring EB resistance could not be determined in this study. However, the likelihood of this high percentage of positional associations to have occurred by chance is remote. Rather, it is indicative of possible involvement of the RGAs with disease resistance in tomato and that the RGAs might be good candidates for resistance genes. Several previous investigations demonstrated linkage associations between genomic locations of RGAs and R genes in different plants including soybean (Kanazin et al. 1996; Yu et al. 1996), potato (Leister et al. 1996), *Arabidopsis* (Aarts et al. 1998; Speulman et al. 1998), lettuce (Shen et al. 1998), maize (Collins et al. 1998), rice (Chen et al. 1998; Mago et al. 1999), and wheat and barley (Chen et al. 1998; Seah et al. 1998). In *Arabidopsis*, for example, two RGAs were actually derived from a functional R gene or from a member of an R gene family (Aarts et al. 1998; Speulman et al. 1998). Thus, the RGA approach has the potential to identify viable candidates for disease resistance loci, including those conferring EB resistance in tomato. However, cloning and molecular characterization of the RGAs are necessary before any functional relationship can be established. Currently, we are sequencing and characterizing the identified RGAs.

Prospects for developing tomatoes with EB resistance using MAS

Similar to that for many other desirable horticultural characteristics in tomato, sources of genes for EB resistance are found only within related wild species. Although the wild species of tomato are cross-compatible with the cultivated types, introgression of genes, in particular those for quantitative traits, is not without inherent difficulties. For example, “linkage drag” is a major barrier particularly when transferring multiple genes. This might be a reason for the limited progress that has been made in developing commercially acceptable tomato cultivars with improved EB resistance. Considering the complexity of EB resistance and the undesirable associations between this trait and other morphological and physiological characteristics (as described earlier), it is highly unlikely that transfer of resistance from the wild accession PI126445 to the cultivated tomato can be accomplished efficiently through conventional protocols of plant breeding. The present study identified several QTLs for EB resistance in PI126445 and determined that a combination of 5–6 complementary QTLs could account for a majority (PVE > 50%) of the total phenotypic variation. Because these QTLs were identified after the elimination of confounding factors, including self-incompatibility, indeterminant growth habit and late-maturity, they should be QTLs with real effects on EB resistance and, thus, of significant value for transferring of resistance from PI126445 to the cultivated tomato via MAS. Introgression of this rather small number of QTLs via MAS is feasible, providing a good prospect for developing commercially acceptable tomato cultivars with EB resistance.

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