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Detection of QTLs associated with shoot wilting and root ammonium uptake under chilling temperatures in an interspecific backcross population from *Lycopersicon esculentum* × *L. hirsutum*

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Abstract The genetic basis for shoot wilting and root ammonium uptake under chilling temperatures was examined in an interspecific backcross (BC_1) population derived from *Lycopersicon esculentum* Mill. cv T5 and wild *Lycopersicon hirsutum* f. *typicum* accession LA1778. The chilling sensitivity of shoot wilting and ammonium uptake was evaluated in four replicated cuttings from each of 196 BC_1 plants. Wilting was evaluated at two different times: 2 hours (wilting 2 h) and 6 hours (wilting 6 h recovery) after root exposure to 4°C. The BC_1 plants were genotyped with 89 polymorphic RFLP markers, and composite interval mapping was used to detect quantitative trait loci (QTLs). Three QTLs, one each on chromosomes 5, 6 and 9, were detected for wilting 2 h. The presence of a *L. hirsutum* (H) allele at the QTL on chromosomes 5 and 9 decreased wilting, while the H allele at the QTL on chromosome 6 increased wilting. To analyze plant recovery from wilting at 6 h, subsets of the BC_1 population were selected, based on phenotype and genotype, because not all plants wilted at 2 h. The phenotype subset (wilting 6 h-PS) included plants that wilted to a greater degree at 2 h, and the genotype subsets included plants carrying specific allelic compositions at the QTL for wilting 2 h on chromosomes 5 (wilting 6 h-GS-ch5), 6 (wilting 6 h-GS-ch6), and 9 (wilting 6 h-GS-ch9). On chromosome 6, a QTL was located that was associated with three subsets (wilting 6 h-PS, wilting 6 h-GS-ch5 and wilting 6 h-GS-ch9), while on chromosome 7 a QTL was detected with two subsets (wilting 6 h-PS and wilting 6 h-GS-ch5). Three additional QTLs were detected within a single subset: chromosome 1 (wilting 6 h-GS-ch6), chromosome 11 (wilting 6 h-GS-ch5) and chromosome 12 (wilting 6 h-GS-ch9). The presence of the H allele at the QTL on chromosomes 7 and 12 had a positive effect, enhancing recovery from wilting, while the H allele at the other QTL had a negative effect. Three traits were used to evaluate the chilling sensitivity of root ammonium uptake: ammonium uptake before a chilling episode, ammonium uptake after the chilling episode, and the relative inhibition of uptake (difference in uptake rates before and after chilling divided by the rate before chilling). One QTL was detected on chromosome 3 for the rate before chilling and one on chromosome 6 for the relative inhibition of ammonium uptake. Our results demonstrate that shoot wilting and ammonium uptake under chilling are controlled by multiple QTLs.

Key words *Lycopersicon esculentum* · *Lycopersicon hirsutum* · Chilling tolerance · QTL · Shoot wilting

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Introduction

The cultivated tomato (*Lycopersicon esculentum* Mill.) is highly sensitive to low temperatures: growth and development is inhibited under 10°C, and temperatures below 6°C can cause irreparable damage (Geisenberg and Stewart 1986). Wild tomato species have geographic distributions that range from sea level to 3700 m (Warnock 1991). High-altitude ecotypes exhibit tolerance to chilling, surviving temperatures near or below freezing each night (Vallejos 1979).

Genetic variability in wild *Lycopersicon* species provides a valuable source of genes controlling desirable traits for the improvement of cultivated tomato through breeding (Rick 1983). Introgression of genes governing chilling tolerance from wild to cultivated tomato would have practical importance for the following reasons. It could extend the growing season of tomato to increase production over time, adapt tomato to areas with shorter growing seasons, and prevent fruit and plant damage from low temperatures (Rick 1983; Scott and Jones 1986). In addition, irrigation water savings may be realized since rains can be used more effectively for crop production in an extended season (Wolf et al. 1986). Fruit quality may be improved by altering planting dates

to avoid exposure of fruit to high temperatures that are typical of some tomato-producing regions in mid-summer.

Several investigators have attempted to identify characteristics useful in estimating differences in chilling tolerance between the cultivated tomato and its wild relatives. Wolf et al. (1986) compared the rate of seed germination and growth under low temperatures for cultivated and wild tomatoes. Walker et al. (1990) used chlorophyll fluorescence to estimate chilling tolerance in a backcross self (BC_1F_2) population between *L. esculentum* and *Lycopersicon hirsutum* and found segregation for chilling tolerance in the progeny. Vallejos and Pearcy (1987) measured the rate of plant growth with the plastochron index to estimate chilling tolerance in hybrids between the same two species. Kamps et al. (1987) evaluated chilling injury in hybrids between *L. esculentum* and *Solanum lycopersicoides* after 72 h exposure to 2.5°C by using a visual rating of wilting damage and chlorophyll fluorescence. Hybrids of *L. esculentum* and *Lycopersicon peruvianum* were evaluated for chilling tolerance (Brüggemann et al. 1996) using chlorophyll fluorescence.

A few studies have investigated the genetic basis of chilling tolerance in tomato. In a backcross population between *L. esculentum* and *L. hirsutum*, Vallejos and Tanksley (1983) used differential growth at 5°C for 3 weeks as a criterion. Three quantitative loci associated with chilling tolerance were detected on chromosomes 6, 7 and 12 using isozyme markers. In crosses between the same two species, Zamir et al. (1982) conducted pollinations at low temperatures and detected two segments on chromosomes 6 and 12 associated with the selection of pollen containing *L. hirsutum* alleles. Polesskaya et al. (1995) evaluated chilling tolerance using indexes of early maturation, number of flowers per inflorescence and fruit setting in progeny from crosses between chilling-tolerant and chilling-resistant tomato cultivars, and found genetic factors on chromosomes 2, 6 and 7 associated with tolerance. In a BC_1 population between *L. esculentum* and *Lycopersicon pimpinellifolium*, Foolad et al. (1998) mapped two QTLs on chromosomes 1 and 4 conferring cold tolerance during seed germination.

Tolerance to chilling temperatures has been studied in cucumber (Wehner and Smeets 1992) and rice (Nagamine and Nakagahra 1990) using a visual rating of plant wilting under chilling temperatures. This trait was reported to be heritable in cucumber and is most likely controlled by more than one gene (Wehner and Smeets 1992). In segregating generations of rice, this trait was associated with a single gene (Nagamine 1991). Karsay et al. (1997), studying components of winter-hardiness in barley, found one major QTL responsible for cold tolerance in a winter×spring barley double-haploid population.

Smart and Bloom (1991) reported that short exposures to chilling temperatures damaged root NH_4^+ uptake in a cultivated tomato (*L. esculentum* cv T5) but not in *L. hirsutum* LA1778, a high altitude Peruvian accession. By contrast, root NO_3^- uptake recovered quickly from chilling in both species. In a subsequent study, Bloom et al. (1998) analyzed these taxa and their F_1 hybrid for the NH_4^+ absorption before and after a chilling episode. The chilling sensitivity of NH_4^+ absorption exhibited by *L. esculentum* appeared to be dominant in the F_1. During the chilling episode, they also observed severe chilling-induced shoot wilting in *L. esculentum* individuals, but not in *L. hirsutum. L. esculentum* plants were able to recover some turgor by the end of the chilling episode.

In this study, we examine the genetic basis of shoot wilting and root NH_4^+ uptake under chilling temperatures in a backcross (BC_1) population, derived from cultivated tomato *L. esculentum* cv T5 (chilling sensitive) and *L. hirsutum* accession LA1778 (chilling tolerant). Replicated cuttings from each of the 196 BC_1 plants were evaluated for these traits upon exposure of the roots to chilling temperatures and the data used to map quantitative trait loci (QTLs).

Materials and Methods

Plant material

Seeds of *L. esculentum* cv T5 and a *L. hirsutum* f. *typicum* accession LA1778 from a high-altitude site in Peru (3022 m; Smart and Bloom 1988) were obtained from the Tomato Genetic Resource Center at U.C. Davis (Rick and Chetelat 1995). T5 is an autogamous, homozygous inbred line, while LA1778 is self-incompatible, heterozygous and highly heterogeneous. Unilateral incompatibility requires that *L. esculentum* must be used as the pistillate parent in crosses between these two species. Under standard greenhouse conditions, interspecific F_1 hybrid seed was produced by placing pollen from a single *L. hirsutum* LA 1778 individual plant (designated LA1778-HS34) on to emasculated *L. esculentum* cv T5 flower buds. A single, interspecific F_1 hybrid plant was used as the pollen donor in crosses with pistillate T5 to produce seed of the first backcross generation (BC_1) to *L. esculentum*.

Plants from the BC_1 population were grown to maturity in 4-l pots in the greenhouse under standard conditions. In order to obtain repeated measurements of each BC_1 individual, two vegetative cuttings were taken from the 196 mature BC_1 plants at two different times: Spring and Summer of 1998. Young, growing shoot tips were selected to provide actively growing meristemic tissue. Vegetative cuttings were used because self-incompatibility in the BC_1 precluded self-pollination to obtain seed for replicated observations on selfed BC_1 families. The cuttings of the BC_1 population (and parental T5 and LA1778-HS34 as controls) were grown to a size in which the plants had between three and six true leaves and at least 30 mg of root dry biomass. Subsequently, the cuttings were evaluated as described below to obtain four replicated observations per BC_1 plant for the two physiological traits, the chilling responses of shoot wilting and root NH_4^+ uptake.

Linkage map

Southern analysis was performed as described in Williams and St.Clair (1993). Briefly, DNA from both parents and the F_1 hybrid was extracted using the CTAB procedure, digested with five restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I), separated in agarose gels, and blotted onto nylon membranes. Membranes were then hybridized with 105 ^{32}P-labeled tomato cDNA and genomic clones, supplied by S. Tanksley (Cornell University), of known position on the tomato linkage map (Tanksley et al. 1992); a few clones were associated with two loci. Two other

probes of unknown position were used: a potato clone for a tonoplast intrinsic protein possibly related to aquaporins (PotRB7; Heinrich et al. 1996) and a tomato clone for galactinol synthase (Gs-2; supplied by K. Bradford, U.C. Davis). After hybridization, blots were exposed to X-ray film and scored for polymorphism between the parental lines to obtain a set of polymorphic probes. The DNA from 196 individuals of the BC_1 population was genotyped with this set of RFLP probes using the same procedures as above.

A linkage map for the polymorphic RFLP probes was constructed with MAPMAKER/EXP 3.0 software (Lander and Botstein 1987; Lincoln et al. 1992) using a 3.0 LOD score as the threshold. Map units in cM were calculated using the Haldane function. A BC_1 map with 89 markers that had an average spacing of approximately 10–15 cM between markers was used for the subsequent QTL analysis (see section below).

Measurement of physiological traits

Bloom et al. (1998) demonstrated that a brief exposure (6 h) of cultivated tomato roots to chilling temperatures (4°C) decreased root NH_4^+ influx and caused the shoot to wilt. In contrast, *L. hirsutum* exposed to the same treatment showed little change in the NH_4^+ uptake and no wilting.

To measure the degree of chilling-induced shoot wilting and change in root NH_4^+ uptake, vegetative cuttings of 196 BC_1 and parental (T5, LA1778-HS34) plants were grown at root and shoot temperatures of 20°C under hydroponic conditions (Hoagland's solution; Epstein 1972). The plants were randomly assigned to a measurement system with 36 individual chambers (Bloom et al. 1998) the night before an experiment and allowed to recover overnight from any transplant shock. This system allowed us to monitor net NH_4^+ uptake from the 36 chambers simultaneously (Bloom et al. 1998). Nutrient solutions contained 50 μM of $NH_4H_2PO_4$ as the nitrogen source. In the morning, plants were illuminated (PPFD of 600 mol m^{-2} s^{-1} at plant height), beginning 1.5 h before the first uptake measurements took place and continuing through all measurements. The chambers were flushed with 5 vol of fresh solution to compensate for nitrogen depletion during the night, the flow through the chamber was stopped, and then the plants were allowed to deplete the solution in the chamber for 6 min. Net uptake (unchilled NH_4^+ uptake) was calculated as the change in NH_4^+ concentration times the chamber volume divided by the depletion time. After this first measurement, the temperature of the nutrient solution was reduced to 4°C and maintained for 6 h. Plants were scored visually for shoot wilting after 2 h (referred to as wilting 2 h) and 6 h (referred to as wilting 6 h-recovery) under 4°C. A score of 0 corresponded to full shoot turgor (i.e., the *L. hirsutum* phenotype), a score of 1 indicated the leaflet tips were flaccid, a score of 2 indicated that more than 50% of the leaf area was flaccid, and a score of 3 indicated complete wilting of the the leaves (i.e., the *L. esculentum* phenotype). After 6 h at 4°C, the nutrient solution was warmed up to 20°C and then a second NH_4^+ uptake measurement was obtained (referred to as the chilled ammonium uptake). NH_4^+ concentrations in the solution samples were analyzed by HPLC as described previously (Bloom et al. 1998).

Data for all 196 BC_1 plants were considered together for the analysis of the 'wilting 2 h' trait. For 'wilting 6 h-recovery', we used subsets of the data that included only those BC_1 plants that had wilted at 2 h because only plants that had wilted at 2 h could show recovery at 6 h. Data subsets were chosen based on either phenotype or genotype. We selected phenotypically (PS) those individuals with a mean phenotypic value for wilting at 2 h of 1.5 or above (this trait was referred to as 'wilting 6 h-PS'). Once putative QTLs for 'wilting 2 h' were detected (see Fig. 3), we created other subsets of the BC_1 population data based on genotypic selection (GS) for the allelic composition at the markers most significantly associated with the QTLs on chromosomes 5, 6 and 9. First, the data subset 'wilting 6 h-GS-ch5' consisted of individuals with the *L. esculentum* allele (E) at markers TG23 and TG413 on chromosome 5. Secondly, 'wilting 6 h-GS-ch6' consisted of a subset of individuals with the *L. hirsutum* allele (H) at markers potRB7 and TG406-A on chromosome 6. Finally, 'wilting 6 h-GS-ch9' consisted of a subset of individuals with the *L. esculentum* allele (E) at markers TG254 and CT143 on chromosome 9. We could not select for the three QTLs simultaneously because the subset of individuals remaining after selection was too small (less than 50 individuals) for meaningful QTL analysis.

QTL analysis

QTL analysis was performed using the composite interval mapping option in the computer program suite QTL Cartographer (Basten et al. 1994, 1997). Composite interval mapping (Zeng 1993, 1994) was conducted using forward stepwise regression with backward elimination to choose the background markers (model FB in the routine 'Srmapqtl'). We used a window size (i.e., the region of the chromosome on either side of the markers flanking the test site that is not considered in the analysis) of 10 cM and a number of markers to control the genetic background ranging from 5 to 10, depending on the trait. Permutation test analyses were performed to estimate the specific likelihood ratio (LR) thresholds for each of the traits using a 5% significance level and 1000 permutations.

At the flanking markers of a given QTL, the phenotypic mean value for individuals homozygous (EE) or heterozygous (EH) was calculated. A *t*-test ($P \leq 0.05$) was used to detect significant differences between the phenotypic means for each genetic combination of alleles. The phenotypic effects were expressed as a percentage of the difference in mean value between the parental lines (EE–HH) and reflected the change in phenotype due to the substitution of an E allele for an H allele.

Results

Scaffold linkage map

We constructed a linkage map using the genotype data for 112 polymorphic RFLP markers on 196 BC_1 individuals derived from *L. esculentum* cv T5 and *L. hirsutum* LA1778. From this map, we selected 89 RFLP markers distributed across the chromosomes at approximately 10–15 cM intervals. These 89 RFLPs were used to construct a map that covered a total genetic distance of 1053.7 cM (Haldane recombination frequency), with a mean distance between markers of 11.8 cM. The biggest gap between adjacent markers was on chromosome 1 (37.4 cM), with all other gaps smaller than 26.1 cM. The 89-marker map was used for the subsequent QTL analysis.

The order of the markers generally agreed with the marker order of the *L. esculentum* × *L. pennellii* F_2 linkage map of tomato (Tanksley et al. 1992). The only discrepancies between our map and the Tanksley et al. (1992) map were on the distal part of chromosome 6 (markers TG314 and CT206) and on chromosome 10 (markers CD32-B and TG63). Distances between markers were generally in agreement with the previously published map, with slight differences due most likely to marker density differences and the use of genetically diverse parents for the mapping populations.

Marker segregation

Regions that deviated from the expected BC_1 1:1 segregation ratio were found on nine (1, 3, 5, 6, 7, 8, 10, 11,

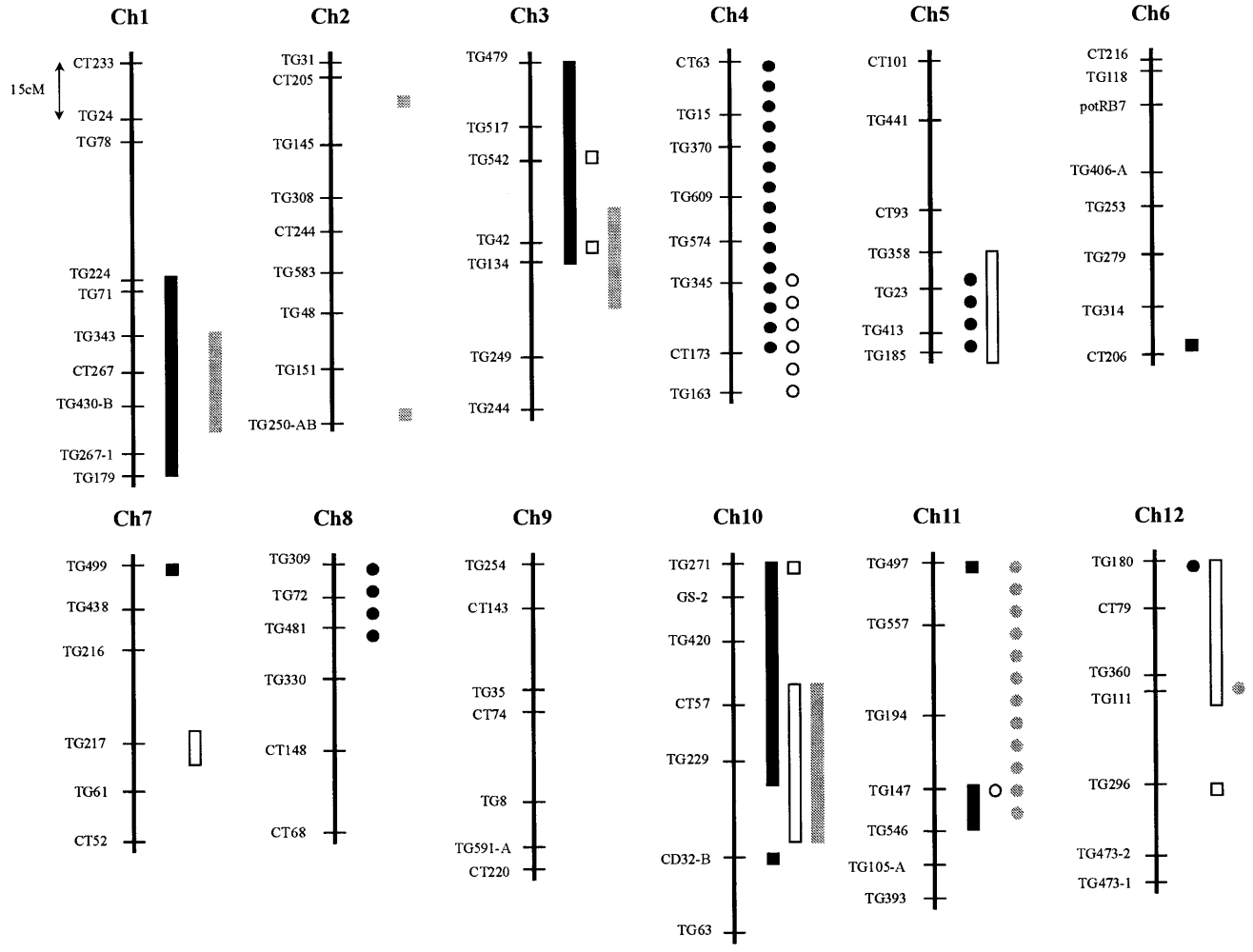

Fig. 1 Distribution of skewed regions in three BC_1 maps from *L. esculentum* × *L. hirsutum*. *Black symbols:* BC_1 *L. esculentum* cv T5 × *L. hirsutum* accession LA1778 (our population). *White symbols:* BC_1 *L. esculentum* cv NC84173 × *L. hirsutum* accession LA2099 (Jones and St.Clair, unpublished data). *Grey symbols:* BC_1 *L. esculentum* cv E6203 × *L. hirsutum* accession LA 1777 (Bernacchi and Tanksley 1997). *Rectangles* indicate distortion towards *L. hirsutum*. *Circles* indicate distortion towards *L. esculentum*. Markers listed are from our BC_1 map. Comparisons were performed using the Tanksley et al. (1992) map as a reference

and 12) of the 12 chromosomes. Of the 41 markers on these chromosomes that deviated from a 1:1 ratio, 26 were distorted towards the *L. hirsutum* (H) allele. Our linkage map was compared to two other BC_1 *L. esculentum* × *L. hirsutum* linkage maps [BC_1 *L. esculentum* cv NC84173 × *L. hirsutum* acc. LA2099 (Jones and St.Clair, unpublished data) and BC_1 *L. esculentum* cv E6203 × *L. hirsutum* acc. LA 1777 (Bernacchi and Tanksley 1997)] in Fig. 1. The distribution of distorted regions was conserved among some chromosomes and linkage maps (chromosomes 1, 3, 4, and 10). The skewed regions were conserved on chromosomes 5, 11, and 12, but the distortion was towards different parental alleles, depending on the cross. Even though 37.3% of the markers showed distorted segregation, the average proportion of *L. esculentum* genome in the population was 75%, as expected for a BC_1.

Chromosome 4 showed maximum distortion for all the markers, except for marker TG163 located at the end of the long arm. The distortion was maximized at marker TG609, located near the heterochromatic centromere region. Only two out of 196 individuals in the BC_1 population were carrying the H allele for this marker. These two individuals had inherited the whole chromosome 4 from *L. hirsutum*.

Chilling-sensitive shoot wilting

The phenotypic frequency distributions of the BC_1 population, parental, and F_1 lines for chilling-induced wilting and recovery were determined (Fig. 2). For wilting 2 h, there was a slight skewing towards the *L. esculentum* parent. For the different subsets of wilting 6 h-recovery, the skewing was towards the *L. hirsutum* parent. The distortion was more pronounced in the genetically selected subsets (described in the Materials and methods section).

Three QTLs associated with wilting 2 h were detected on chromosomes 5, 6, and 9 (Fig. 3; Table 1). The *L. hirsutum* allele (H) at the QTLs on chromosomes 5 and 9

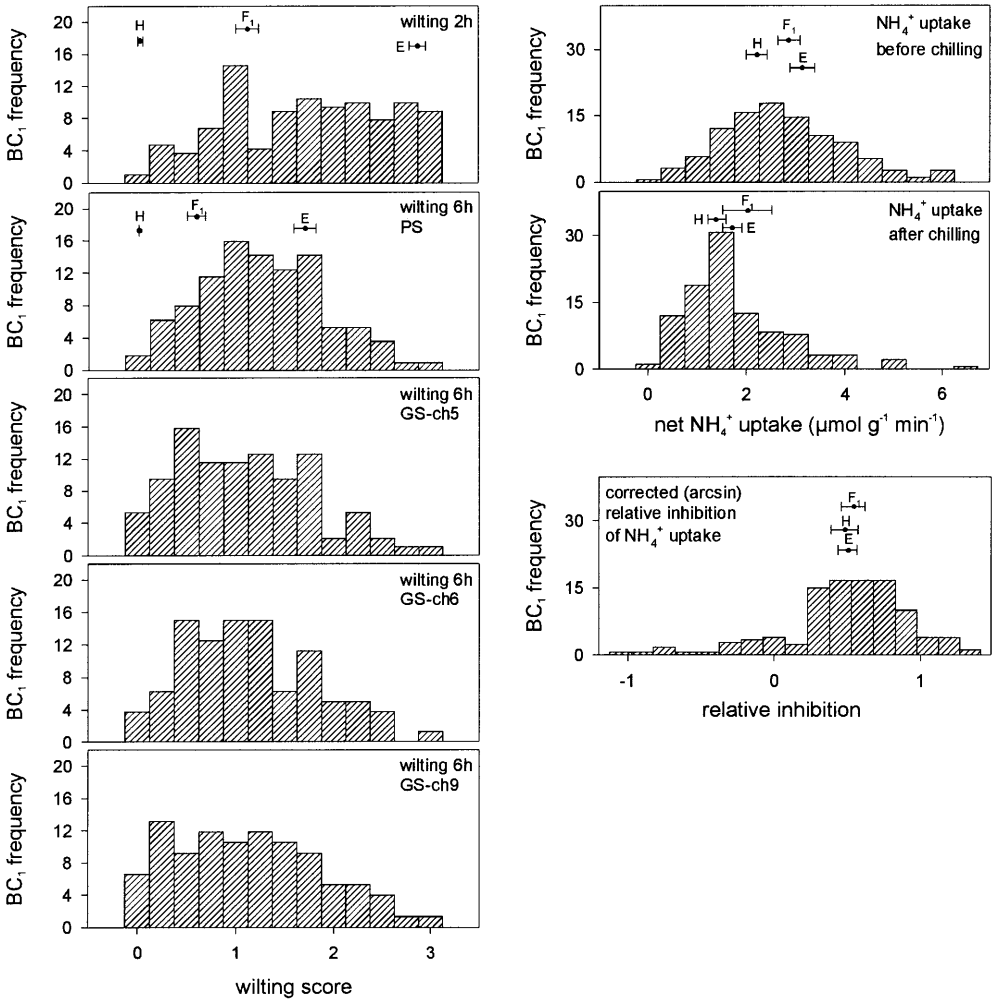

Fig. 2 Frequency distribution for all traits (wilting 2 h, wilting 6 h recovery, ammonium uptake) considered in this study. E is the mean value for the *L. esculentum* parent, F_1 is the mean value for the F_1 hybrid, and H is the mean value for the *L. hirsutum* parent

had a positive additive effect (Table 2); the presence of the H allele in a plant decreased wilting. The QTL on chromosome 6 had a negative additive effect (Table 2) in that the H allele at this position increased the wilting score of individuals carrying it. The QTL on chromosome 9 had the largest phenotypic effect, explaining 33% of the total variation of the trait, and exhibited the largest likelihood-ratio test statistic of all the QTLs detected (Table 1). The mean phenotypic values for wilting 2 h for individuals carrying either the *L. esculentum* (E) or H allele at the flanking marker positions of the putative QTLs were calculated (see Table 2). All *t*-tests comparing the mean phenotypic values for E and H for the three QTLs were highly significant ($P \leq 0.01$).

The QTL analysis for the wilting 6 h-recovery data revealed a more complex situation than for wilting 2 h. For the phenotypically selected subset wilting 6 h-PS, two QTLs on chromosomes 6 and 7 were detected (Fig. 3; Tables 1, 2). The QTL on chromosome 7 had a positive additive effect and explained approximately 28% of the variation. In contrast, the QTL on chromosome 6 had a negative additive effect in that the H allele increased the wilting score and the mean phenotypic value of individuals with the H allele was significantly higher than those carrying the E allele (Table 2). The QTL on chromosome 7 was only significant at marker TG217, the marker closer to the highest significant point of the QTL (Fig. 3).

With the genotypically selected subset for the QTL on chromosome 5, wilting 6 h-GS-ch5, the same two QTLs on chromosomes 6 and 7 that were associated with wilting 6 h-PS were detected, plus two additional QTLs located on chromosomes 9 and 11 (Tables 1, 2; Fig. 3). The QTL on chromosome 11 had a negative additive effect, with the H allele decreasing the recovery from chilling-induced wilting. The QTL on chromosome 9 was associated with the same two markers as the QTL detected for wilting 2 h (Fig. 3). When comparing the phenotypic values at flanking markers for the QTLs in chromosomes 7 and 9 (Table 2), only one of the two flanking markers was significant, TG217 for chromosome 7 and CT143 for chromosome 9.

For wilting 6 h-GS-ch6, the QTL on chromosome 9 was also detected, with an additional QTL on chromosome 1 (Tables 1, 2; Fig. 3). This second QTL had a negative additive effect (Table 2) and was only significant ($P \leq 0.05$) at marker TG78, the marker closest to the highest significant value of the QTL (Fig. 3). For wilting 6 h-

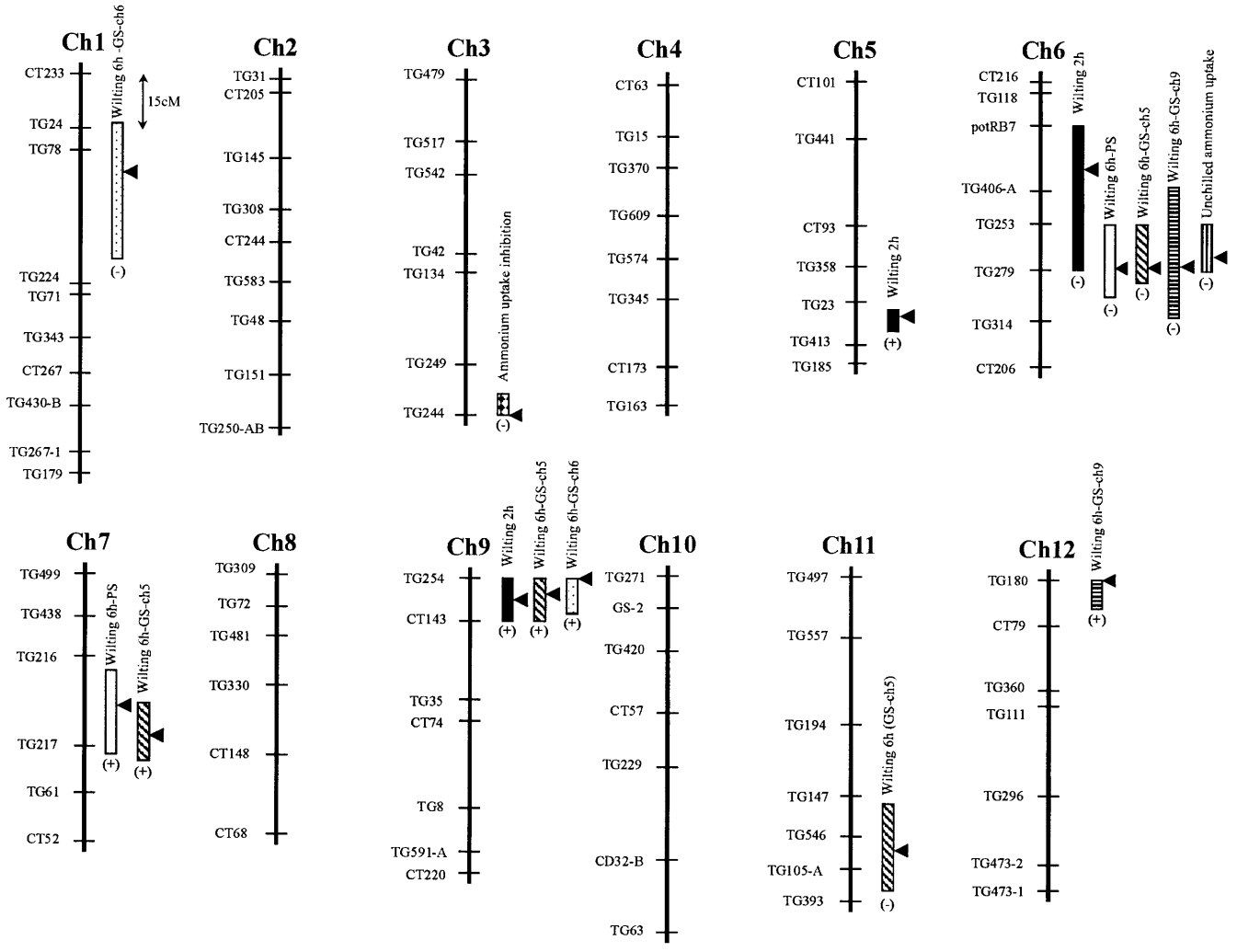

Fig. 3 Linkage map and QTL positions. *Arrows* indicate the highest significant value of the QTL. The positive (decrease in shoot wilting score or decrease in chilling inhibition of NH_4^+ uptake) or negative (increase in shoot wilting score or increase in chilling inhibition) additive effect of a specific QTL is indicated by a '+' or a '–' under the QTL bars, respectively

GS-ch9, two QTLs on chromosomes 6 and 12 were mapped. Only the QTL on chromosome 12 had a positive additive effect. The QTL on chromosome 6 extended along the chromosome between markers TG406-A and TG314 (Fig. 3), coinciding in part with the QTL detected for wilting 2 h. The phenotypic mean value of individuals with the E allele at the QTL on chromosome 6 was significantly lower than the mean of individuals with the H allele.

Ammonium uptake before and after chilling

The frequency distributions of the three NH_4^+-uptake traits (Fig. 2) revealed that NH_4^+ uptake after chilling was skewed towards the *L. hirsutum* parent. The non-normal distribution for the relative inhibition of NH_4^+ uptake was corrected using the arcsin transformation. Mean values for the parents (T5, LA1778) were calculated from cuttings evaluated at the same time as the BC_1 population. However, previously published results (Bloom et al. 1998) showed a larger difference between *L. esculentum* cv T5 and *L. hirsutum* accession LA1778 for the relative inhibition of NH_4^+ uptake under chilling than observed in our BC_1 experiment (Fig. 2).

The QTL analysis for these three traits revealed the presence of two QTLs (Tables 1, 2; Fig. 3). A QTL for relative inhibition of NH_4^+ uptake was located at the terminal portion of chromosome 3 and had a negative effect in that the H allele from *L. hirsutum* produced an increase in chilling inhibition. The QTL on chromosome 6 for NH_4^+ uptake before chilling also had a negative effect on the phenotype. The QTL on chromosome 6 was located at the same position as the QTL for wilting 6 h-recovery (see Fig. 3). The phenotypic mean values were significantly different for both QTLs (Table 2).

Table 1 Results of the QTL analysis for each trait. Shoot-wilting traits are listed under 'A' and NH_4^+-uptake traits under 'B'. The QTL threshold was calculated by permutation analysis for each trait

Trait	# Ind.	# Markers/ window (cM)[a]	Threshold (0.05)	QTL							
				Ch1	Ch3	Ch5	Ch6	Ch7	Ch9	Ch11	Ch12
A											
Wilting 2 h	196	10/10	13.2			TG23-TG413[b] 14.4[c] 8.5[d]	potRB7-TG279 30.6 21.2		TG254-CT143 65.4 33.3		
Wilting 6 h PS	113	10/10	13.3				TG253-TG314 22 18.1	TG216-TG61 19.9 27.7			
Wilting 6 h GS-ch5	91	10/10	13.3				TG253-TG314 19.7 18.8	TG216-TG61 14.6 16.2	TG254-CT143 22.7 23.8	TG147-TG393 16.3 17.9	
Wilting 6 h GS-ch6	80	8/10	14	CT233-TG224 26.8 37.2					TG254-CT143 25.1 26.9		
Wilting 6 h GS-ch9	79	10/10	13.8				TG406A-TG314 43.9 43.9				TG180-CT79 23.5 27.1
B											
Unchilled ammonium uptake	196	5/10	12.4				TG253-TG279 30 17.5				
I nhibition ammonium uptake	196	6/10	13		TG249-TG244 16.4 8.6						

[a] The # markers indicate the number of markers used to control for the genetic background on the composite interval mapping. 'Window' indicates the window size in cM at both sides of the QTL that is blocked during the analysis
[b] Markers flanking a QTL
[c] Likelihood ratio test statistic for H_0:H_1. To convert LR to LOD values, LOD=0.217 LR
[d] Percentage of the phenotypic variance explained by the QTL

Table 2 Phenotypic values at the QTL positions and QTL effects. Shoot-wilting traits are listed under 'A' and NH_4^+-uptake traits under 'B'

Trait	QTL position	Marker interval[a]	Phenotypic mean value[b]		*t* test[c]	Effect[d]
			E	H		
A						
Wilting 2 h	ch5	TG23	1.857	1.454	0.0014**	+14.4
		TG413	1.871	1.441	2.9×10^{-4}***	+15.4
	ch6	potRB7	1.490	1.849	0.0025**	–12.9
		TG406-A	1.476	1.892	4.6×10^{-4}***	–14.9
	ch9	TG254	2.022	1.336	1.8×10^{-9}***	+24.6
		CT143	2.094	1.324	8.3×10^{-11}***	+27.6
Wilting 6 h-PS	ch6	TG279	0.899	1.436	1.3×10^{-5}***	–31.9
	ch7	TG216	1.322	1.103	0.0968 n.s.	+13.0
		TG217	1.326	1.056	0.0261*	+16.1
Wilting 6 h-GS-ch5	ch6	TG279	0.755	1.283	1.9×10^{-4}***	–31.4
	ch7	TG216	1.177	0.910	0.0874 n.s.	+15.9
		TG217	1.260	0.828	0.0026**	+25.7
	ch9	TG254	1.145	0.940	0.1445 n.s.	+12.2
		CT143	1.203	0.889	0.0296*	+18.7
	ch11	TG546	0.881	1.140	0.0775 n.s.	–15.4
		TG105-A	0.984	1.136	0.2965 n.s.	–9.0
Wilting 6 h-GS-ch6	ch1	TG78	0.815	1.387	5.8×10^{-5}***	–34.0
		TG224	0.903	1.222	0.0584 n.s.	–19.0
	ch9	TG254	1.397	0.858	1.9×10^{-4}***	+32.1
		CT143	1.312	0.894	0.0059**	+24.9
Wilting 6 h-GS-ch9	ch6	TG279	0.728	1.491	2.4×10^{-6}***	–45.4
	ch12	TG180	1.222	0.901	0.0623 n.s.	+19.1
		CT79	1.175	0.948	0.1952 n.s.	+13.5
B						
Trait	QTL position	Marker interval[a]	Phenotypic mean value[b]		*t* test[c]	Effect[d]
			E	H		
Unchilled Ammonium Uptake	ch6	TG253	2.170	3.022	3.8×10^{-5}***	–23.3
		TG279	2.012	3.087	7.0×10^{-8}***	–29.5
Inhibition Ammonium Uptake	ch3	TG249	0.360	0.533	0.0116*	–61.8
		TG244	0.339	0.568	6.2×10^{-4}***	–81.8

[a] Marker interval that includes the highest significant level on the QTL. When only one marker is considered, it indicates that the highest significant value of the QTL is located at the exact marker position
[b] Phenotypic mean value of individuals carrying the *L. esculentum* allele (E) or the *L. hirsutum* allele (H)
[c] Results for the *t* test for the phenotypic mean values, where * $P\leq0.05$, ** $P\leq0.01$, *** $P\leq0.001$
[d] Effect of substituting an E allele by an H allele on the phenotype was calculated as [(E-H)/P]*100, where P is the difference between phenotypic values of the two parental lines. A "+" positive effect indicates a decrease in the wilting score or a decrease in the chilling inhibition of ammonium uptake and a "–" negative effect indicates an increase in the wilting score or an increase in the chilling inhibition

Discussion

Linkage map

Deviation from the expected Mendelian segregation ratios has been reported in a variety of interspecific tomato populations (Helentjaris et al. 1986; Paterson et al. 1988; de Vicente and Tanksley 1993; Grandillo and Tanksley 1996). Populations differ for the degree of distortion and the location of the distorted regions on the chromosomes. Such variation was evident when we compared three different BC_1 populations derived from *L. esculentum* and *L. hirsutum* (Fig. 1). The degree of distortion ranged from 15% in a population generated by Bernacchi and Tanksley (1997) to 37.3% in our population. These values are similar to values of 34% and 25% in F_2 populations from crosses between *L. esculentum* and *L. hirsutum* (Helentjaris et al. 1986; Maliepaard et al. 1995).

The location of distorted regions appeared to be conserved for some of the chromosomes, and some regions seemed to be consistently distorted towards the wild parent (Fig. 1). Chromosomes 3 and 10 contained regions skewed towards the *L. hirsutum* (H) allele in all three BC_1 *L. esculentum* × *L. hirsutum* populations, agreeing also with the results of Maliepaard et al. (1995) for chromosome 10. The long arm of chromosome 1 showed distortion towards the H allele, agreeing with the distortion reported in an interspecific cross with *L. pimpinellifollium* (Grandillo and Tanksley 1996). In a F_2 population from a cross between *L. esculentum* and *L. pennellii*, 66% of the markers were distorted towards the wild parent (de Vicente and Tanksley 1993). This consistency of distortion across populations may indicate that

these regions carry genes for which the wild allele is selected under similar experimental conditions (e.g., greenhouse environment, temperature conditions, etc.). On the other hand, some chromosomes did not exhibit consistent patterns of distortion. For example, the skewness on chromosome 11 is conserved, but towards different alleles in different populations. Almost no distortion appeared in the three BC_1 populations for chromosome 7 (Fig. 1), in contrast to the severe distortion observed by Maliepaard et al. (1995). This disparity may indicate that minute differences between the specific parent lines were responsible for the location, presence, and magnitude of the distortions.

The severe distortion encountered on chromosome 4 in our study (i.e., 196 out of 198 BC_1 individuals carried the E allele at marker TG 609 on chromosome 4, and severe skewing towards E was observed at most other markers on this chromosome) should be considered from a different perspective. Tomato and other species contain a locus, gamete eliminator (*Ge*), that causes the selective abortion of gametes carrying a specific allele due to allelic interaction (Rick 1966; Sano 1990). *Ge* has been suggested as a mechanism for maintaining reproductive barriers among plant species since it has been detected mostly in interspecific hybrids (Sano 1990). In tomato, three alleles are known at this locus, Ge^n, Ge^c and Ge^p, but only gametes carrying the Ge^c allele are eliminated in Ge^c/Ge^p hybrids (Rick 1970). The locus *Ge* in tomato is located in the vicinity of *ful* and *w-4*, in the heterochromatic centromeric region of chromosome 4 (Rick 1970). A comparison of the classical map (Khush and Rick 1968) and the molecular linkage map of tomato (Tanksley et al. 1992) suggests that for all the markers segregating for chromosome 4 in our population, TG609 is the closest to the position of *Ge*. Maximum distortion was observed for this marker (frequency of E alleles=0.99), suggesting that selection against gametes carrying the H allele from *L. hirsutum* in the F_1 hybrid contributed to the severe distortion in the BC_1.

QTL detection in tomato interspecific crosses has not been reported to be adversely affected by marker distortion. QTLs for various traits have been mapped in chromosomal regions containing distorted and non-distorted markers (de Vicente and Tanksley 1993; Goldman et al. 1995; Bernacchi and Tanksley 1997). We observed that distorted markers were associated with QTLs on chromosomes 5, 11 and 12, but not with QTLs on chromosomes 1, 3, 6, 7, and 9 (Figs. 1, 3).

Chilling-sensitive shoot wilting QTLs

Phenotypic evaluations of shoot wilting due to chilling were divided into two groups: wilting that appeared 2 h after the roots reached 4°C (wilting 2 h) and the degree of recovery after 6 hours at 4°C (wilting 6 h). For wilting 2 h, three QTLs were detected on chromosomes 5, 6, and 9 (Fig. 3; Tables 1, 2). The H allele at the QTLs on chromosomes 5 and 9 reduced wilting, as expected based on the *L. hirsutum* phenotype, but the H allele at the QTL on chromosome 6 increased wilting, a negative phenotypic effect. Phenotypic effects of parental alleles opposite to those predicted on the basis of the parental phenotype have been reported previously in tomato (de Vicente and Tanksley 1993; Bernacchi et al. 1998). Previous studies on chilling tolerance in tomato have shown genetic factors associated with markers located on chromosome 6. Some markers were associated with a negative effect on growth under chilling (Vallejos and Tanksley 1983) and others had a positive effect on pollen fertilization under cold temperatures (Zamir et al. 1982).

Recovery from chilling-induced wilting QTLs

Two QTLs were detected on chromosomes 6 and 7 for recovery from wilting after 6 h exposure to chilling temperatures (Fig. 3; Tables 1, 2). The QTL on chromosome 6 was detected with all data subsets (except wilting 6 h-GS-ch6 that removed the effect of the chromosome-6 QTL), with the highest peak of the QTL coinciding with the position of marker TG279. TG279 has been reported to be tightly linked to the *sp* locus (Paterson et al. 1988; Grandillo and Tanksley 1996). The *sp* locus, for self-pruning (i.e., determinancy), controls growth habit. MacArthur (1932) first described determinancy as a recessive character. In contrast to the indeterminant (*Sp*-) growth habit in which the inflorescences are regularly spaced at every third node, the inflorescence appears at every node or every second node in the determinant (*spsp*) phenotype, resulting in limited growth of the shoot, a bushy plant habit, and more concentrated fruit set. Our BC_1 population was segregating 1:1 for determinant growth habit, and showed a positive association between the presence of the E allele at TG279 and the ability to recover from wilting. In determinant *spsp* homozygotes, there is a change in the regularity of the occurrence of inflorescences and a reduction in the internode length (Pnueli et al. 1998). Certain other characteristics, like fruit size and fruit soluble solids, are also affected by growth habit (Emery and Munger 1970).

A QTL on chromosome 7 was detected in the analysis for wilting 6 h-PS and for wilting 6 h-GS-ch5, yet the *t*-tests of the mean phenotypic values were only significant at TG217, one of the two flanking markers (Table 2). This may be due to the smaller population size for this analysis (97 individuals), or the fact that the highest peak for the QTL was closer to TG217 (Fig. 3). The QTL on chromosome 7 differed from the QTL on chromosome 6 in that the H allele from *L. hirsutum* conferred a higher rate of recovery from wilting, a positive effect. Vallejos and Tanksley (1983) also detected genetic factors on chromosome 7, linked to isozyme loci *Got-2* and *Got-3*, with a positive effect of the H allele on growth at low temperatures of BC_1 plants derived from *L. esculentum* × *L. hirsutum*. TG217 is close to the position of *Got-2* on the tomato map (Tanksley et al. 1992), suggesting that the same genetic factor(s) may be acting under chilling conditions in our population.

The QTL analyses of data subsets permitted the detection of additional QTLs. The main effect of removing individuals carrying specific alleles at QTLs for wilting 2 h from the population was that additional QTLs were detected that were not significant (below the threshold) with the phenotypically selected data set (Tables 1, 2). The additional QTLs were located on chromosome 1 for wilting 6 h-GS-ch6, chromosome 11 for wilting 6 h-GS-ch5 and chromosome 12 for wilting 6 h-GS-ch9. The H allele had a positive effect on the phenotype by increasing recovery from wilting only at the QTL on chromosome 12, although the differences in mean phenotypic values were not significant. Studies on pollen fertilization (Zamir et al. 1982) and growth (Vallejos and Tanksley 1983) in backcross populations of *L. esculentum* × *L. hirsutum* under low temperatures showed that genetic factors controlling these traits were associated with markers located on chromosome 12. However, this region did not appear to coincide with the QTL we detected. In QTL studies on cold tolerance during seed germination, Foolad et al. (1998) reported a QTL on chromosome 1 in the same region as the QTL we detected for wilting 6 h-GS-ch6. The QTL on chromosome 9 for wilting 6 h-GS-ch5 and wilting 6 h-GS-ch6 is most likely a residual effect from the QTL on chromosome 9 for wilting-2 h. This chromosome-9 QTL only appears when the individuals carrying the E allele for the QTL on chromosome 5 or the H allele for the QTL on chromosome 6 are removed from the analysis.

Ammonium uptake under chilling QTLs

The chilling sensitivity of root ammonium uptake in tomato has been reported by Smart and Bloom (1991) and Bloom et al. (1998). These two studies showed a clear distinction in root NH_4^+ uptake after a chilling episode between *L. esculentum* (chilling sensitive) and *L. hirsutum* (chilling tolerant), with *L. hirsutum* having a higher uptake than *L. esculentum*. In our investigation with a BC_1 population derived from *L. esculentum* × *L. hirsutum*, the results for the QTL analysis of the NH_4^+ uptake traits were not what we expected. Even though the trait was segregating in the BC_1 population (Fig. 2), we detected two QTLs in which the H allele contributed to decreased NH_4^+ uptake after chilling, an effect opposite to that of the *L. hirsutum* phenotype. Previous QTL studies in tomato have confirmed that allelic effects are sometimes opposite to that of the parental phenotype. Ammonium uptake appears to be highly influenced by the environment, contributing to low trait heritability. Our QTL analysis was performed on the means of four replicated cuttings per BC_1 plant, while Bloom et al. (1998) used more than 20 replications per genotype to detect significant differences between *L. esculentum*, *L. hirsutum*, and their F_1 hybrid. The utilization of 20 replications for each of the 196 BC_1 plants for QTL mapping is a prohibitively large experiment. Obviously, additional replications and a larger population size would increase the ability to detect QTLs for NH_4^+ uptake and might reveal additional QTLs for shoot wilting.

Summary

The traits shoot wilting and root NH_4^+ uptake under chilling temperatures were found to be controlled by multiple QTLs in an interspecific backcross population. Chilling-induced shoot wilting is controlled by at least three QTLs, and recovery from wilting by at least four QTLs. A minimum of two QTLs are involved in ammonium uptake. Future studies will address the genetic and physiological effects of specific QTLs in a uniform *L. esculentum* background, and the potential use of these QTLs in breeding chilling-tolerant cultivated tomato. We are currently using marker-assisted selection during backcrossing to transfer the H allele at the QTLs on chromosomes 5 and 9 for decreased shoot wilting under chilling into *L. esculentum* to produce genetic stocks. These stocks will be used to study the phenotypic effects of each QTL, alone and in combination, and to assess their value for breeding.

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