Theor Appl Genet (2005) 111: 898–905
DOI 10.1007/s00122-005-0015-2

ORIGINAL PAPER

F. John Goodstal · Glenn R. Kohler · Leslie B. Randall
Arnold J. Bloom · Dina A. St.Clair

A major QTL introgressed from wild *Lycopersicon hirsutum* confers chilling tolerance to cultivated tomato (*Lycopersicon esculentum*)

Received: 8 April 2005 / Accepted: 9 June 2005 / Published online: 2 August 2005

Abstract Many plants of tropical or subtropical origin, such as tomato, suffer damage under chilling temperatures (under 10°C but above 0°C). An earlier study identified several quantitative trait loci (QTLs) for shoot turgor maintenance (stm) under root chilling in an interspecific backcross population derived from crossing chilling-susceptible cultivated tomato (*Lycopersicon esculentum*) and chilling-tolerant wild *L. hirsutum*. The QTL with the greatest phenotypic effect on stm was located in a 28 cM region on chromosome 9 (designated *stm9*), and enhanced chilling-tolerance was conferred by the presence of the *Lycopersicon hirsutum* allele at this QTL. Here, near-isogenic lines (NILs) were used to verify the effect of *stm9*, and recombinant sub-NILs were used to fine map its position. Replicated experiments were performed with NILs and sub-NILs in a refrigerated hydroponic tank in the greenhouse. Sub-NIL data was analyzed using least square means separations, marker-genotype mean *t*-tests, and composite interval mapping. A dominant QTL controlling shoot turgor maintenance under root chilling was confirmed on chromosome 9 using both NILs and sub-NILs. Furthermore, sub-NILs permitted localization of *stm9* to a 2.7 cM interval within the original 28 cM QTL region. If the presence of the *L. hirsutum* allele at *stm9* also confers chilling-tolerance in *L. esculentum* plants grown under field conditions, it has the potential to expand the geographic areas in which cultivated tomato can be grown for commercial production.

Communicated by R. Hagemann

F. John Goodstal · G. R. Kohler · L. B. Randall
A. J. Bloom · D. A. St.Clair (✉)
Department of Plant Sciences, University of California-Davis, Mail Stop 3, One Shields Avenue, Davis, CA 95616-8780, USA
E-mail: dastclair@ucdavis.edu
Tel.: +1-530-7521740
Fax: +1-530-7529659

Introduction

Cultivated tomato, *Lycopersicon esculentum* Mill. (synonym *Solanum lycopersicum*; Spooner et al. 2005), like many other crop plants of topical or subtropical origin, is sensitive to chilling as well as freezing temperatures. Temperatures under 10°C inhibit tomato growth, and those under 6°C can cause irreparable damage (Geisenberg and Stewart 1986). In contrast, the interfertile wild species *Lycopersicon hirsutum* Dunal (synonym *S. habrochaites*; Spooner et al. 2005) grows at altitudes up to 3,300 m in the Peruvian Andes and thrives under chilling conditions (Vallejos 1979; Bloom et al. 2004).

Introgression of genes controlling chilling tolerance from wild congeners into *L. esculentum* has potential to improve resistance to chilling temperatures. Several practical benefits of increased chilling tolerance in cultivated tomato would be: extension of the growing season, which would increase production over time; adaptation of tomato to areas with shorter growing seasons; and prevention of plant and fruit damage from chilling temperatures (Rick 1983; Scott and Jones 1986). Another possible benefit would be a decreased need for irrigation if rain were available during an extended growing season (Wolf et al. 1986).

Several hypotheses have been proposed to explain tolerance or sensitivity to chilling in plants. One hypothesis is that the transition of cellular membranes from a fluid to a gel phase at chilling temperatures impairs membrane permeability (Lyons and Raison 1970). A second is that chilling inhibits energy metabolism or stimulates the production and/or accumulation of deleterious byproducts (Guy 1994). A third is that chilling temperatures markedly decrease root hydraulic conductance, causing chilling-sensitive species to suffer water stress (Wilson 1976; Markhart et al. 1979; Bagnall et al. 1983; Fennel and Markhart 1998; Aroca et al. 2001; Bloom et al. 2004). Our current research focuses on this third hypothesis.

A number of phenotypic assessment criteria have been used in studies of the genetic control of chilling tolerance in tomato. Vallejos and Tanksley (1983) used the plastochron index as a measure of chilling tolerance after several weeks at low temperatures. Wolf et al. (1986) employed the rate of seed germination and growth under low temperatures to compare cultivated and wild tomatoes. A shift in chlorophyll fluorescence under chilling exposure has been used by several researchers (Kamps et al. 1987; Walker et al. 1990; Brüggemann et al. 1994). We used the degree of shoot wilting after an episode of root chilling (Truco et al. 2000; Bloom et al. 2004). The current study also uses this criterion.

Several studies have explored the genetic basis of chilling tolerance in tomato. In a BC_1 population derived from *L. esculentum* cv. T3 × *L. hirsutum* acc. LA1777, three quantitative trait loci (QTLs) controlling the plastochron index were detected on chromosomes 6, 7, and 12 (Vallejos and Tanksley 1983). In another study, growth at suboptimal temperatures (15°C day/10°C night) of interspecific progeny derived from a cross of *L. esculentum* cv. T5 and a primitive *L. esculentum* cultivar, which exhibited cold tolerance during seed germination, were compared to T5 after 5 weeks (Foolad and Lin 2001). The primitive cultivar accumulated three times more shoot biomass than T5 under suboptimal temperatures. Analysis of multiple generations derived from this cross showed that growth under suboptimal temperatures was genetically controlled by at least two genes that are predominantly additive in nature.

Truco et al. (2000) identified several QTLs controlling shoot wilting under root chilling in an interspecific BC_1 *L. esculentum* cv. T5 × *L. hirsutum* acc. LA1778 mapping population. The QTL accounting for the largest percent of the phenotypic variation (33%) was located on chromosome 9 and was designated *stm9* for shoot turgor maintenance. A segregating BC_1S_1 population derived from a selected plant from this mapping population also showed an association between the presence of *L. hirsutum* alleles at *stm9* and a significantly faster relative growth rate after chilling (A.J. Bloom et al., unpublished data). In the present study, we further localize the position of *stm9* through the use of near-isogenic lines (NILs) and recombinant sub-NILs for chromosome 9 and confirm its effect on shoot turgor maintenance under root chilling conditions.

Materials and methods

Plant material

The interspecific BC_1 mapping population, described in Truco et al. (2000), was created by crossing *L. esculentum* cv. T5 (T5) with *L. hirsutum* acc. LA1778 (individual plant selection HS34), followed by a backcross of one selected F_1 (individual plant selection HY34-5) to T5 to obtain BC_1 seed. The BC_1 population was planted in 1998 and consisted of 196 individuals. Subsequently, a single selected BC_1 plant (BC_1-33) was used as the pollen donor to generate both the homozygous and heterozygous NILs. Subsequently, marker assisted selection (MAS) was used during two generations of backcrossing to pistillate parent T5 (BC_2 and BC_3) for introgression of the *L. hirsutum* allele at the QTL on chromosome 9, originally detected by Truco et al. (2000). Here we refer to this QTL for shoot turgor maintenance on chromosome 9 as *stm9*. BC_1-33 was selected as the *stm9* QTL donor due to a high percentage of *L. esculentum* background alleles outside the QTL region as well as a favorable growth habit. NILs for *stm9* were selected from one heterozygous BC_3 plant (03GH_230) by selfing and marker-selecting individuals in the BC_3S_1 generation. Plants selected as paired NILs were either homozygous for *L. hirsutum* (*H*/*H*) or *L. esculentum* (*E*/*E*) alleles at all markers in the *stm9* region (see "MAS for NILs"). To generate recombinant sub-NILs at *stm9*, one heterozygous (*E*/*H*) BC_3 plant (03GH_230) was backcrossed to T5 and the resulting BC_4 population was marker-screened for those individuals showing recombination in the *stm9* QTL interval (see "MAS for sub-NILs").

MAS for NILs

To create NILs for QTL *stm9*, MAS was performed during two successive generations of backcrossing to T5 (BC_2 and BC_3). The QTL region associated with *stm9* was ~12 cM in Truco et al. (2000). To ensure capture of the entire *L. hirsutum* chromosomal segment associated with the stm phenotype, the region subjected to MAS for the present study was expanded to ~28 cM with additional markers on both sides of the original ~12 cM region. A population of 100 BC_2 plants was foreground-selected for individuals heterozygous (*E*/*H*) in the *stm9* region with the two markers flanking this QTL (Truco et al. 2000): TG254, a PCR based Cleaved Amplified Polymorphic (CAP) marker (Konieczny and Ausubel 1993), and CT143, a Restriction Fragment Length Polymorphic (RFLP) marker. In addition, six CAP markers and 23 RFLP markers were used in background selection for *E* alleles outside the target *stm9* region. Tomato genomic and cDNA clones for RFLP markers of known position on the tomato linkage map (Tanksley et al. 1992; http://www.sgn.cornell.edu) were used according to procedures described by Truco et al. (2000). A single BC_2 plant (01GH_4507) that was heterozygous at *stm9* and at three regions outside the QTL (top of chromosome 1, and top and bottom of chromosome 2) but homozygous for T5 alleles throughout the rest of the genome (as detected with the available markers) was selected for backcrossing to T5 to obtain the BC_3 population. A population of 310 BC_3 plants was foreground-selected for individuals heterozygous for markers at *stm9* (Table 1) with two flanking CAP

Table 1 Tomato CAPS markers used in MAS to develop near-isogenic lines for QTL *stm9* on chromosome 9

Markers	Marker Primers (forward and reverse primer combinations)	Restriction enzyme	Anneal Temp.(°C)
cLPT4c24	TCT GAA AAA GGG TCT CTG TTC ATC TTT GGC AAT AGT CTT ATC CTG GTC	*Hpy188 III*	51
TG254	ATG AGG GAA TAT GGT CAT GGT TAC GAA ATG TGG AAC TGG TTG AGG TAG	*Apo I*	57
T1641	CAA ACT AGT GCA CAA TTC CAA AAC AAA GAG CAA CAA TAT CTG CAC AAG	*Dde I*	53
T1670	ATT CAA GTG GAA CCA ATA CAT GG AAT CAT GCA GCA CTT GGA ATA TC	*Hinf I*	53
T1673	CTA ATT TTA TTG AAG CCT CAG ATG G AAA TCA TCA ACT CAT CCA CCA TAG	n/a	53
T532	AAG GTT AAC CAA ATC GGT AGT GTG AGT CTG CTT GTT ATT TCA CCA AGG	*Hinf I*	53
TG223	GAT GGT TGA GTG ATT GAA GC TTG AAC ACA CTG TAA TCC CC	*Mse I*	60
T1617	GTG AAC TCT ACA ACA GAG CCA AAC GTT TTC CTC TCT CCA TTA CCA TTG	*Hae III*	53

For each pair of primers, the optimal annealing temperature was determined by empirical testing. PCR reactions were performed on a PE9700 thermal cycler (Perkin Elmer, Boston, MA, USA). PCR was performed with an initial denaturation of 94°C for 2 min, followed by 35 cycles of 94°C for 45 s, annealing temp for 45 s, and 72°C for 60 s, with a final extension of 72°C for 2 min. After digestion with the appropriate restriction enzyme (NEB, Beverly, MA, USA), PCR products were separated on a 2.0% agarose gel

markers, cLPT4c24 and T1617, and one internal Sequence Characterized Amplified Region (SCAR) marker, T1673 (http://www.sgn.cornell.edu). For background selection, three CAP and 14 RFLP markers were used. A single BC_3 plant (03GH_230) heterozygous at markers for *stm9* and homozygous for T5 alleles throughout the rest of the genome was selected for creation of the BC_3S_1 and BC_4 populations. A population of 79 BC_3S_1 plants was foreground-selected for individuals homozygous for either *L. esculentum* (*E*/*E*) or *L. hirsutum* alleles (*H*/*H*) at *stm9* with the same three markers as before (cLPT4c24, T1617, and T1673). Twelve BC_3S_1 individuals, six homozygotes from each *stm9* genotypic class (*E*/*E* or *H*/*H*), were selected as NILs (Table 2) for phenotypic evaluation.

MAS for sub-NILs

A population of 628 BC_4 individuals derived from BC_3 donor 03GH_230 were genotyped with three markers cLPT4c24, T1617, and T1673 to detect recombinants in the *stm9* region. Thirty-five unique and independent recombinants were found and further genotyped with an additional five CAP markers (TG254, T1641, T1670, T532 and TG223) within *stm9*. Twenty-five recombinant sub-NILs, divided into 14 sub-NIL genotypic class groups (designated A through N), were selected for phenotypic evaluation. Each sub-NIL genotypic class group contained one to four lines (Fig. 1). Individuals heterozygous (*E*/*H*) and homozygous (*E*/*E*) throughout the *stm9* region were also selected in the BC_4 to serve as NIL controls. CAP marker T532 replaced RFLP marker CT143 reported in Truco et al. (2000) and is located 0.2 cM away. Marker spacing in the *stm9* region ranged between 1.7 to 7.7 cM apart and averaged one marker every ~4 cM over the ~28 cM QTL region represented in the recombinant sub-NILs.

Linkage map

The linkage map for the top of chromosome 9 (Figs. 1 and 2) was based on a T5 × LA1778 BC_1 population

Table 2 Stmscore means for *stm9* BC_3S_1 NILs and control lines from the NIL experiment

Generation	Line	Class	stmscore	Lsmeans seperation
Parental	*L.esc*.T5	E/E	2.71	A
BC_3S_1	03GH_1303	E/E	2.22	A B
BC_3S_1	03GH_1331	E/E	2.13	A B
BC_3S_1	03GH_1306	E/E	1.59	A B C
BC_3S_1	03GH_1304	E/E	1.51	A B C
BC_3S_1	03GH_1310	E/E	1.42	A B C
BC_3S_1	03GH_1318	E/E	1.39	A B C D
BC_3	03GH_230	E/H	0.87	B C D E
BC_3S_1	03GH_1329	H/H	0.75	B C D E
BC_4	03GH_47	E/H	0.68	C D E
BC_4	03GH_48	E/H	0.61	C D E
BC_3S_1	03GH_1321	H/H	0.39	D E
BC_3S_1	03GH_1375	H/H	0.36	D E
BC_3S_1	03GH_1350	H/H	0.30	D E
BC_4	04GH_60	E/H	0.26	D E
BC_1	$BC_1$33	E/H	0.23	E
BC_3S_1	03GH_1322	H/H	0.09	E
BC_3S_1	03GH_1305	H/H	0.06	E
BC_2	01GH_4507	E/H	0.05	E
Parental	*L.hir*.HS34	H/H	0.04	E

Class column indicates the genotype (*E*/*E*,*E*/*H* or *H*/*H* alleles) for all markers in the *stm9* region for each line. Stmscore values shown are back-transformed least-square (LS) means. LSmeans separation was performed using the Tukey-Kramer method adjusted for multiple comparisons; line means that share a letter in common do not differ significantly at $P \leq 0.05$

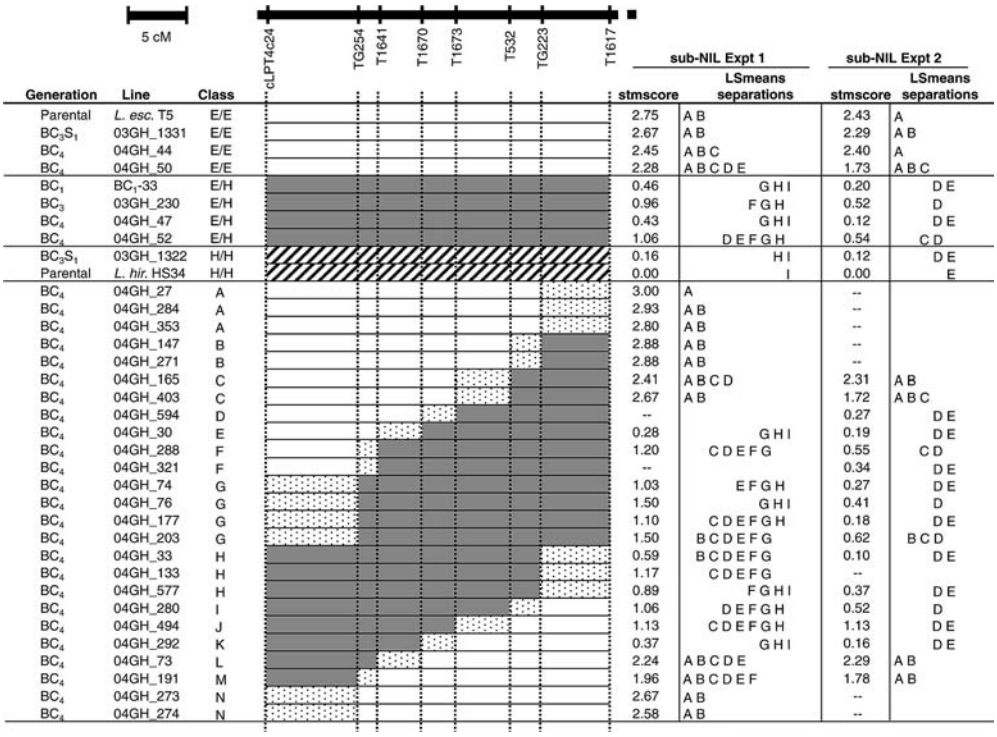

Fig. 1 Graphical genotypes and stmscore means for *stm9* BC_4 sub-NILs and control lines from sub-NIL experiments 1 and 2. Class column indicates the genotype (*E*/*E*, *E*/*H* or *H*/*H* alleles) for all markers in the *stm9* region in the control lines or the sub-NIL recombinant genotypic class group (see Materials and methods). For graphical genotypes, *white horizontal bars* indicate homozygous for *L. esculentum* alleles, *diagonal hatched bars* indicate homozygous for *L. hirsutum* alleles, *grey* indicates heterozygosity, and *stippled bars* indicate marker intervals containing a recombination. Stmscore values shown are back-transformed least-square (LS) means. LSmeans separations were performed using the Tukey-Kramer method adjusted for multiple comparisons; line means that share a letter in common do not differ significantly at $P \leq 0.05$

containing 400 individuals (196 individuals used by Truco et al. 2000, plus an additional 204 individuals, unpublished data) that were genotyped with 12 DNA markers (3 RFLP and 9 CAP) on chromosome 9. The linkage map was created with Mapmaker/QTL version 3.0 using the Haldane recombination frequency and LOD ≥3.0 (Lincoln et al. 1992). Five of the 12 markers were ≤ 0.2 cM from another marker, and so were not included in the figures.

Phenotyping

Vegetative shoot tip cuttings (~14.0 cm in length) were obtained for each individual genotype (sub-NIL, NIL or control). All cuttings were dipped in Rootone, a rooting compound that contains auxin and a fungicide (GardenTech, USA), and allowed to root in a vermiculite/perlite mixture for 1 week in the greenhouse. Cuttings were grown and phenotyped in the same greenhouse, located at UC Davis. During the experiments, ambient greenhouse temperatures were 25–37°C day, 18–25°C night and relative humidity was 55–80% day, 20–55% night. Cuttings were watered daily and fertilized with a 28:14:14 solution on the second and forth day after planting. After 1 week, cuttings were transferred to hydroponic growth tanks located in the greenhouse. The tanks contained modified Hoagland's solution (Epstein and Bloom 2005) at 21°C, and the plants were allowed to grow for at least 1 week under ambient illumination. The cuttings were then randomized, moved into a separate refrigerated hydroponic tank containing the same nutrient solution, and allowed to acclimate overnight at a root temperature of 21°C. The following morning, under supplemental lighting from one or two 1,000 W metal halide lamps to maintain the light level (PFD) above 1,000 μmol m^{-2} s^{-2} PAR, the tank temperature was lowered to 5–6°C, and held there for 2 h before phenotyping. Cuttings were phenotyped for 'stmscore,' a measure of shoot turgor maintenance under root chilling, on a 0 to 3 scale (0 = completely turgid shoot, 1 = leaflet tips were flaccid, 2 = more that 50% of the leaf area was flaccid and 3 = completely wilted).

Experimental design

Since a comparison among NILs or sub-NILs was desired, the NIL and two sub-NIL experiments were each conducted as a randomized complete block design (RCBD). The NIL experiment consisted of five replications carried out between March 9 and 25, 2004. Sub-NIL experiment 1 consisted of eight replications carried out between April 5 and 16, 2004 and sub-NIL experiment 2 consisted of nine replications carried out between May 10 and 21, 2004. The NIL

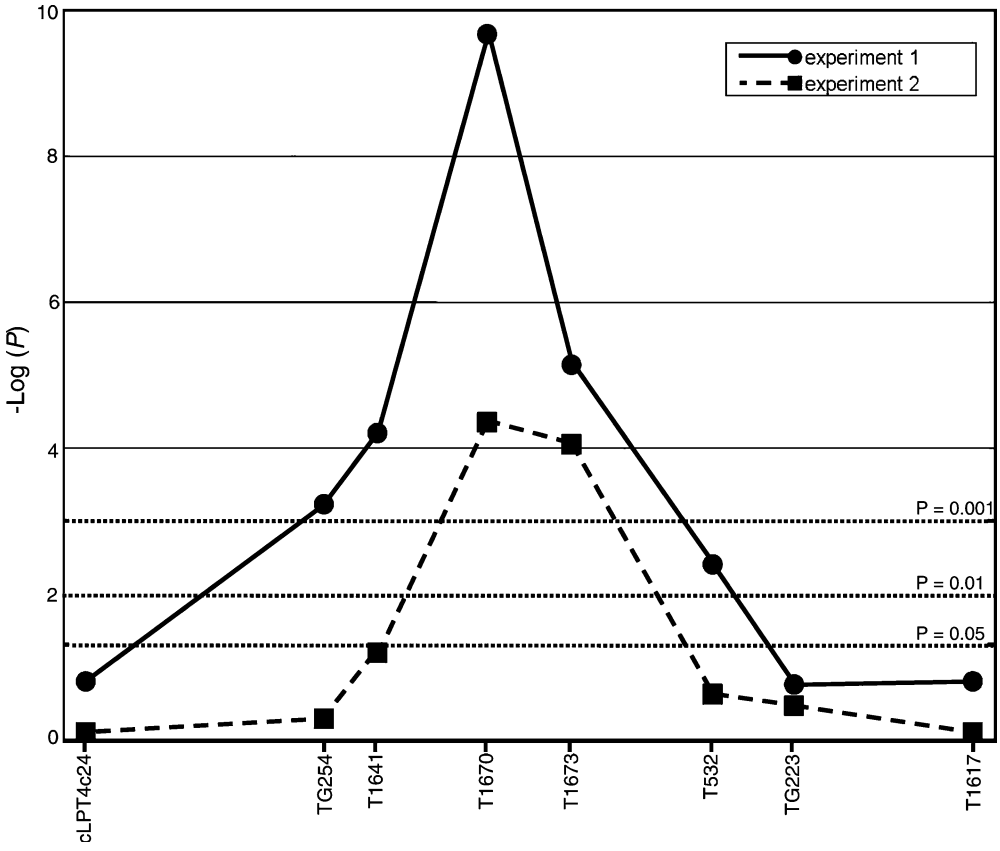

Fig. 2 Significance [plotted as $-\log(P)$] of marker-genotype mean *t*-test comparisons among *stm9* BC_4 sub-NILs from experiments 1 and 2. Mean stmscores are plotted separately for each experiment. Significance thresholds at $P = 0.05$, 0.01 and 0.001 are indicated by their respective *dashed lines*

and sub-NIL experiments had the following parental controls included in each replication: four cuttings of chilling-susceptible *L. esculentum* cv. T5, two cuttings of chilling-tolerant genotype BC_1-33, and two cuttings of chilling-tolerant *L. hirsutum* acc. LA1778-HS34 (Table 2, Fig. 1). The NIL experiment also contained two cuttings of chilling-tolerant BC_2 genotype 01GH_4507 per replication (Table 2). The sub-NIL experiments included additional NIL controls per replication, consisting of four cuttings of chilling-susceptible homozygous genotype (*E*/*E*) at *stm9* region, (one cutting each of BC_4 lines 04GH_44 and 04GH_50, and two cuttings of BC_3S_1 line 03GH_1331), four cuttings of chilling-tolerant heterozygous genotype (*E*/*H*) at *stm9* (two cuttings of BC_3 line 03GH_230, and one cutting each of BC_4 lines 04GH_47 and 04GH_52) and two cuttings of chilling-tolerant homozygous genotype (*H*/*H*) at *stm9* (BC_3S_1 line 03GH_1322).

The NIL experiment included six NILs *H*/*H* at *stm9* and six NILs *E*/*E* at *stm9*. Both sub-NIL experiments 1 and 2 included sub-NIL class groups C, and E – M (Fig. 1). The first sub-NIL experiment also included sub-NIL classes A, B and N, which were excluded from the second experiment, based on previous results from experiment 1 (Fig. 1). There was insufficient material available for sub-NIL class D and line 04GH_321 from class F for the first experiment, and line 04GH_133 from class H for the second experiment. The number of cuttings used varied between the two sub-NIL experiments. In the first, one cutting was used for each line for all sub-NIL classes. In the second, one to two cuttings were used per line, depending on the number of lines present in the sub-NIL class; a single cutting was used for lines which had more than one representative in its sub-NIL class (classes C, F, G, and H); all other classes contained one line each, so two cuttings per line were included per replication (sub-NIL classes D, E, I–L, and M).

Statistical analysis and QTL mapping

The data for all three experiments were analyzed separately as RCBD using analysis of variance (ANOVA) via a general linear model (PROC GLM, version 8.1, SAS Institute, 1999). To test the assumptions of ANOVA, we used PROC UNIVARIATE (SAS 8.1) to calculate the Shapiro–Wilk test for normality (Shapiro and Wilk 1965), Levene's test for homogeneity of error variance (Levene 1960), and Tukey Single Degree of Freedom for Non-Additivity test (Tukey 1949). To meet these assumptions, it was necessary to remove several outlying data points in each experiment and reduce heterogeneity of error variance by transformation. The NIL experiment required transform $(\text{stmscore} + 2)^{-1/2}$, sub-NIL experiment 1 used transform $(\text{stmscore} + 2)^{1/2}$, and sub-

NIL experiment 2 required transform (stmscore + 2)$^{-2}$ to meet ANOVA assumptions. Least square means (LSmeans) were calculated from transformed data and used in all subsequent analyses. The localization of *stm9* was accomplished by identifying individual NILs that maintained shoot turgor to a greater extent than chilling-sensitive T5 according to LSmeans separation adjusted for multiple comparisons (Tukey–Kramer method) at $P \leq 0.05$ (Table 2).

Fine mapping of *stm9* with sub-NILs was performed using three methods. First, at each marker locus, phenotypic data for lines homozygous for cultivated *L. esculentum* (*E*/*E*) or heterozygous for *L. hirsutum* (*E*/*H*) were grouped, and then the phenotypic means for the *E*/*E* and *E*/*H* genotype groups were compared using two-sample *t*-test calculated by PROC GLM of SAS (SAS Institute 1999). The *t*-test *P*-values for marker-genotype comparisons were graphed as $-\log(P)$; thus the most likely QTL position is indicated by peaks (Brouwer and St.Clair 2004). Significance thresholds at $P = 0.05$, 0.01 and 0.001 are indicated on Fig. 2. Secondly, evidence for the location of *stm9* was also obtained by identifying individual sub-NILs that had lower stmscores than chilling-sensitive T5 according to the LSmeans separation adjusted for multiple comparisons (Tukey–Kramer method) at $P \leq 0.05$ (Fig. 1). Third, to verify the results from the LSmeans separations and marker-genotype mean *t*-tests, and to calculate the additive effect of allelic substitution (*H* for *E*) at each marker, QTL analysis was performed on each sub-NIL experiment using composite interval mapping (CIM) (Zeng 1994). CIM was executed using the composite interval mapping option in the software suite Windows QTL Cartographer version 2 (http://statgen.ncsu.edu/brcwebsite/software_BRC.php; Basten et al. 1999) using the forward-backward regression routine, two markers to control background, a 10 cM window, and 1,000 permutations to obtain likelihood ratio (LR) thresholds to declare QTL significance at $P \leq 0.05$, as described in Truco et al. (2000).

Results

Separate ANOVAs were performed for each of the three experiments (NIL, sub-NIL experiment 1 and 2). Data for the first and second sub-NIL experiments were analyzed separately due to variation in both the sub-NIL genotypic classes included and the number of cuttings included per line for each replication. The ANOVAs for the three experiments indicated that replication and line effects were highly significant ($P \leq 0.01$, data not shown). LSmeans separation was performed for the NIL experiment (Table 2) and individually for each sub-NIL experiment (Fig. 1). Significant ($P \leq 0.05$) differences for stmscore between individuals of the *stm9* genotypic class *E*/*E* versus *E*/*H* and *H*/*H* individuals were observed in all three experiments, but *E*/*H* and *H*/*H* genotypic classes did not differ significantly (Fig. 1 and Table 2). When NILs *H*/*H* at *stm9* were compared to NILs *E*/*H* at *stm9* or chilling-tolerant sub-NILs, no significant differences for stmscore were observed in any of the three experiments, implying that the *H* allele at *stm9* exhibits dominance.

Least-square mean comparisons among sub-NILs indicated that lines heterozygous (*E*/*H*) at marker T1670 were chilling-tolerant (as indicated by significantly lower stmscores) in both experiments, suggesting that this marker is associated with *stm9* (Fig. 1). Experiment 2 contained a recombinant individual not included in experiment 1 (class D, line 04GH_594). This chilling-tolerant individual was *E*/*E* for marker T1670 and contained a recombination between markers T1670 and T1673, indicating that *stm9* is located in the 2.7 cM region between these two markers. Marker-genotype mean *t*-tests for each sub-NIL experiment also implied that *stm9* is located between markers T1670 and T1673 (Fig. 2). QTL analysis of each sub-NIL experiment with CIM also indicated that this interval is the most likely position for *stm9*. The stmscore QTL peak LR value/threshold LR value obtained for sub-NIL experiments 1 and 2 were 47.2/9.2 and 43.1/8.9, respectively, with the peak centered on marker T1670 in experiment 1 and in the T1670—T1673 interval in experiment 2 (data not shown). In addition, the additive effect of substitution of the *E* allele with an *H* allele at markers T1670 and T1673 was 1.70 and 1.65 for experiments 1 and 2, respectively.

In summary, all three experiments confirmed the presence of a QTL affecting shoot turgor maintenance under root chilling on chromosome 9. Furthermore, three different data analysis methods—LSmeans separations, marker-genotype *t*-tests, and CIM—identified the same 2.7 cM interval between markers T1670 and T1673 as the most likely location of *stm9*.

Discussion

In our original BC_1 *L. esculentum* cv. T5 × *L. hirsutum* acc. LA1778 QTL mapping study on chilling tolerance (Truco et al. 2000), a major QTL on chromosome 9 controlling shoot turgor maintenance under root chilling (*stm9*) was detected between markers TG254 and CT143 (replaced by T532 in the present study). Here we used a set of NILs and sub-NILs for the *stm9* region to confirm the positive phenotypic effect of the *hirsutum* allele at this QTL and fine map *stm9*. Our experiments verified that this region is strongly associated with shoot turgor maintenance under root chilling (Table 2, Figs. 1, 2). The chromosomal position of this QTL was further refined using sub-NILs and localized *stm9* to a 2.7 cM region between markers T1670 and T1673 (Figs. 1, 2, CIM results).

The genetic control of chilling tolerance in tomato has been studied by several research groups, including Vallejos and Tanksley (1983), Foolad et al. (2001), and

Truco et al. (2000). The first two groups showed that chilling tolerance is controlled by multiple QTLs working in an additive fashion. In contrast, Truco et al. (2000) found a single major and several minor QTLs. Our present study confirms the results of Truco et al. (2000), namely that *stm9* is a major effect QTL, sufficient alone to significantly affect shoot turgor maintenance under root chilling. In a separate study of a segregating BC_1S_1 population (generated from BC_1-33), we also observed a significantly faster relative growth rate after chilling for *E*/*H* and *H*/*H* genotypes at the *stm9* region when compared to *E*/*E* genotypes (A. J. Bloom et al., unpublished data).

NILs and sub-NILs for the *stm9* region made it possible to further detail the contribution of the *hirsutum* allele at *stm9* to the chilling-tolerant phenotype in an otherwise uniform *L. esculentum* genetic background. The use of sub-NILs to fine map and verify a QTL has been shown previously (Paterson et al. 1990; Eshed and Zamir 1995; Bernacchi et al. 1998; Brouwer and St.Clair 2004). Here we localized *stm9* to a 2.7 cM region, much smaller than the originally mapped region (Truco et al. 2000). Further experiments with these sub-NILs for *stm9* will help to elucidate the mechanisms and loci involved in plant responses to cold soils. In future studies using recombinants derived from these sub-NILs, and genomic resources such as tomato BAC and EST libraries, it should be possible to high-resolution map *stm9* and identify candidate loci underlying this QTL that control shoot turgor maintenance and relative growth rates under root chilling (Alpert and Tanksley 1996; Yano et al. 2000; Fridman et al. 2002; Liu et al. 2002).

Physiological characterization of *L. esculentum* cv. T5 and *L. hirsutum* acc. LA1778 offers a possible hypothesis for how shoot turgor maintenance affects chilling tolerance (Bloom et al. 2004). Water flow through the xylem of both T5 and LA1778 was markedly reduced when the roots were chilled. The stomata of *L. hirsutum* closed rapidly when roots were chilled; whereas *L. esculentum* stomata were slow to respond to the decreased water flow from the roots and subsequently the leaves wilted, causing plant damage. Bloom et al. (2004) hypothesized chemical signals from roots to shoots control stomatal behavior, and evidence for this was obtained using grafted tomato plants with combinations of roots and shoots from differing genotypes at *stm9* (*E*/*H* or *E*/*E*) that conferred non-wilting (nw) or wilting (w) shoot phenotypes under root chilling, respectively. Grafted plants subjected to root chilling that had the shoot/root combinations w/nw and nw/w exhibited intermediate stmscores compared to chilling-susceptible w/w and chilling-resistant nw/nw grafted plants, suggesting root-shoot signaling involvement. The sub-NILs for *stm9* can be employed to help elucidate the chemical signals involved in shoot turgor maintenance and its relation to chilling tolerance.

Future studies will also investigate the response of these NILs and sub-NILs in field experiments to determine what effect *stm9* may have in enabling cultivated tomato to survive, and possibly thrive, under cool field conditions earlier or later in the growing season. Improving the chilling tolerance of cultivated tomato in the field is of practical importance for several reasons. Chilling tolerant tomatoes would permit an extended growing season, increasing productivity, and areas that currently have too limited a season for tomato production could be employed (Rick 1983; Scott and Jones 1986). In addition, chilling damage to tomato plants and fruits may be minimized or avoided, reducing production losses. The chilling tolerance at *stm9* shown in our current study is conferred by a dominant allele from the *L. hirsutum* donor. If this dominant chilling-tolerant phenotype is exhibited in field experiments, the introgression of *stm9* would be straightforward and its presence would only be required in one of the inbred parents of an F_1 hybrid cultivar. Most modern tomato cultivars used in production are F_1 hybrids that contain alleles for monogenic disease and pest resistances from wild *Lycopersicon* species (Park et al. 2004); the addition of abiotic stress tolerance would be valuable for further germplasm improvement.

Acknowledgements This research was supported in part by a United States Department of Agriculture National Research Initiative 'Plant Responses to the Environment' grant 00-35100-9530 to AJB and DAS.

References

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

Aroca R, Tognoni F, Irigoyen JJ, Sanches-Diaz M, Pardossi A (2001) Different root low temperature response to two maize genotypes differing in chilling sensitivity. Plant Physiol Biochem 39:1067–1073

Bagnall D, Wolfe J, King RW (1983) Chill-induced wilting and hydraulic recovery in mung bean plants. Plant Cell Environ 6:457–464

Basten CJ, Weir BS, Zeng ZB (1999) QTL cartographer. Version 1.13. North Carolina State University, Raleigh, NC

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

Bloom AJ, Zwieniecki MA, Passioura JB, Randall LB, Holbrook NM, St. Clair DA (2004) Water relations under root chilling in a sensitive and tolerant tomato species. Plant Cell Environ 27:971–979

Brouwer DJ, St.Clair DA (2004) Fine mapping of three quantitative trait loci for late blight resistance in tomato using near isogenic lines (NILs) and sub-NILs. Theor Appl Genet 108:628–638

Brüggemann W, Klaucke S, Linger P, Lindhout P (1994) Quantitative physiological screening parameters for chilling tolerance in tomato breeding. Plant Physiol 105:14

Epstein E, Bloom AJ (2005) Mineral nutrition of plants: principles and perspectives, 2nd edn. Sinauer Associates, Sunderland

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the

identification and fine mapping of yield-associated QTL. Genetics 141:1147–1162
Fennel A and Markhart AH (1998) Rapid acclimation of root hydraulic conductivity to low temperature. J Exp Bot 49:879–884
Foolad MR, Lin GY (2001) Genetic analysis of cold tolerance during vegetative growth in tomato, *Lycopersicon esculentum* Mill. Euphytica 122:105–111
Fridman E, Liu YS, Carmel-Goren L, Gur A, Shoresh M, Pleban T, Eshed Y, Zamir D (2002) Two tightly linked QTLs modify tomato sugar content via different physiological pathways. Mol Genet Genomics 266:821–826
Geisenberg C and Stewart K (1986) Field crop management. In: Atherton JG, Rudich J (eds) The tomato crop: a scientific basis for improvement. Chapman and Hall, London, pp 511–557
Guy CL (1994) Low temperature and crop yield. In: Boote KJ, Bennet JM, Sinclair TR, Paulsen GM (eds) Physiology and determination of crop yield. SSSA, ASA, CSSA, Madison, pp 417–424
Kamps TL, Isleib TG, Herner RC, Sink KC (1987) Evaluation of techniques to measure chilling injury in tomato. HortSci 22:1309–1312
Konieczny A, Ausubel FM (1993) A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4:403–410
Levene H (1960) Robust tests for equality of variances. In: Olkin I (ed) Contributions to probability and statistics: essays in honor of Harold Hotelling. Stanford University Press, Stanford, pp 278–292
Lincoln S, Daly M, Lander ES (1992) Constructing genetic maps with MAPMAKER/EXP 3.0, 3rd edn. The Whitehead Institute Technical Report
Liu J, van Eck J, Cong B, Tanksley SD (2002) A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. Proc Natl Acad Sci USA 99:13302–13306
Lyons JM and Raison JK (1970) Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury. Plant Physiol 45:386–389
Markhart AH, Piscus EL, Naylor AW, Kramer PJ (1979) Effect of temperature on water and ion transport in soybean and broccoli systems. Plant Physiol 64:83–87
Park YH, West MAL, St.Clair DA (2004) Evaluation of AFLPs for germplasm fingerprinting and assessment of genetic diversity in cultivars of tomato (*Lycopersicon esculentum* L.). Genome 47:510–518
Paterson AH, DeVerna JW, Lanini B, Tanksley SD (1990) Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes in an interspecific cross of tomato. Genetics 124:735–742
Rick CM (1983) Genetic variability in tomato species. Plant Mol Biol Rep 1:81–87
SAS Institute (1999) The SAS System for Windows. SAS Online-Doc, version 8. SAS Institute, Cary, North Carolina, USA
Scott SJ, Jones RA (1986) Cold tolerance in tomato. II. Early seedling growth of *Lycopersicon* spp. Physiol Plant 66:659–663
Shapiro SS and Wilk MB (1965) An analysis of variance test for normality (complete samples). Biometrika 52:591–611
Spooner DM, Peralta IE, Knapp S (2005) Comparison of AFLPs with other markers for phylogenetic inference in wild tomatoes [*Solanum* L. section *Lycopersicon* (Mill.) Wettst.] Taxon 54:43–61
Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160
Truco MJ, Randall LB, Bloom AJ, St.Clair DA (2000) Detection of QTLs associated with shoot wilting and root ammonium uptake under chilling temperatures in an interspecific backcross population from *Lycopersicon esculentum* × *L. hirsutum*. Theor Appl Genet 101:1082–1092
Tukey JW (1949) One degree of freedom for non-additivity. Biometrics 5:232–242
Vallejos CE (1979) Genetic diversity of plants for response to low temperatures and its potential use in crop plants. In: Lyons JM, Graham D, Raison JK (eds) Low temperature stress in crop plants. Academic, New York, pp 247–489
Vallejos CE, Tanksley SD (1983) Segregation of isozyme markers and cold tolerance in an interespecific backcross of tomato (*Lycopersicon esculentum* × *Lycopersicon hirsutum*). Theor Appl Genet 66:241–247
Walker MA, Smith DM, Pauls KP, McKersie BD (1990) A chlorophyll fluorescence screening test to evaluate chilling tolerance in tomato. HortSci 25:334–339
Wilson JM (1976) Mechanism of chill-hardening and drought-hardening of *Phaseolus vulgaris* leaves. New Phytol 76:257–270
Wolf S, Yakir D, Stevens MA, Rudich J (1986) Cold temperature tolerance of wild tomato species. J Am Soc Hort Sci 111:960–964
Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y, Sasaki T (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS*. Plant Cell 12:2473–2483
Zeng Z-B (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468