Chilling-induced water stress: Variation in shoot turgor maintenance among wild tomato species from diverse habitats[1]

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- *Premise of the study:* Cultivated tomato, *Solanum lycopersicum*, suffers chilling induced wilting because water movement through its roots decreases with declining soil temperatures. Certain wild tomato species exhibit resistance to chilling-induced wilting, but the extent of this chilling tolerance in wild tomatoes is not known.
- *Methods:* We measured shoot wilting during root chilling in wild *Solanum* accessions from habitats differing in elevation, temperature, and precipitation. We also measured shoot wilting during root chilling in introgression lines (ILs) with chromosome 9 segments collinear to the shoot turgor maintenance QTL *stm9* region from chilling-tolerant *S. habrochaites*, chilling and drought-tolerant *S. lycopersicoides,* or drought-tolerant *S. pennellii.*
- *Key results:* Wild tomato species, which experience chilling temperatures (<10°C) in their native habitat, maintain shoot turgor under root chilling. Among accessions of *S. lycopersicum* var. *cerasiforme*, a typically chilling sensitive species, shoot turgor maintenance during root chilling was correlated with the precipitation of the native habitat. By contrast, *S. pennellii*, a species that is typically drought adapted, did not maintain turgor under root chilling. Grafted plants with roots containing *S. habrochaites* an*d S. lycopersicoides* introgressions improved shoot turgor maintenance under root chilling.
- *Conclusions:* Resistance to chilling-induced water stress is an important adaptation to chilling temperatures in wild tomatoes. There is some overlap in adaptation to drought and chilling stress in some tomato species. Root-based resistance to chilling-induced water stress in wild tomatoes may involve orthologous gene(s).

Key words: adaptation; chilling; drought; elevation; root; Solanaceae; *Solanum*; tomato.

Tomato species (*Solanum* section *Lycopersicon*) originate from South America and grow as herbaceous, facultative perennials in diverse climate zones, ranging from the tropics of the Amazon basin to the arid desert along the coast to the frigid high-altitude Andes (Rick and Yoder, 1988; Smith and Peralta, 2002; Young et al., 2002; Nakazato et al., 2010). Cultivated tomato, *Solanum lycopersicum,* which originates from the wet tropics of South America, is a classic example of a chilling-sensitive crop (Rick, 1976). Exposure of cultivated tomatoes to chilling temperatures (under 10°C but above 0°C) severely inhibits growth and causes significant injury (Geisenberg and Stewart, 1986). Most studies of chilling injury focus on shoot responses to shoot chilling such as membrane permeability (Wilson, 1976; Markhart et al., 1979; Bagnall et al., 1983; Fennell and Markhart, 1998; Aroca et al., 2001; Bloom et al., 2004), photosynthesis and photodamage (Yakir et al., 1986; Vallejos and Pearcy, 1987; Venema et al., 2000). Chilling damage also derives in part from water stress in cold soils because chilling impedes root water absorption, which can result in shoot wilting if stomata do not close rapidly to limit water loss (Wilson, 1976; Markhart et al., 1979; Bagnall et al., 1983; Fennell and Markhart, 1998; Aroca et al., 2001; Bloom et al., 2004).

Shoots of chilling-sensitive *S. lycopersicum* wilt if their roots are exposed to chilling temperatures of around 6°C; by contrast, shoots of high elevation, chilling-tolerant *S. habrochaites* accessions maintain turgor under the same treatment (Vallejos and Pearcy, 1987; Truco et al., 2000; Bloom et al., 2004; Goodstal et al., 2005). This shoot turgor maintenance during root chilling does not derive from higher root hydraulic conductance in *S. habrochaites*, but from stomatal closure during root chilling (Bloom et al., 2004). The sensitivity of stomata to chilling-induced water deficits in *S. habrochaites* is most likely an adaptive response to its frigid habitat and may be important in the adaptation of other wild tomatoes to cold soils and other types of water stress.

A major quantitative trait locus (QTL) *stm9* controlling shoot turgor maintenance under root chilling was mapped on chromosome 9 in an interspecific backcross population derived from *S. lycopersicum* and chilling-tolerant *S. habrochaites* accession LA1778 (Truco et al., 2000). QTL *stm9* was subsequently fine-mapped to a 2.7 cM region (Goodstal et al., 2005). Other wild tomato species also exhibit this trait, which is likely to have a common genetic basis because of the conservation of gene function and DNA marker order (i.e., collinearity) in *Solanum* species in the section *Lycopersicon* (Labate et al., 2007); http://www.solgenomics.net). If regions collinear to *stm9* are also associated with shoot turgor maintenance during root chilling

in cold-tolerant *S. lycopersicoides* and drought-tolerant *S. pennellii*, then root chilling and drought may share regulatory pathways important for general plant responses to abiotic stress.

In the present study, we conducted a survey of *Solanum* accessions including diverse wild tomato species originating from warm, cold, wet, or dry climates to determine whether shoot turgor maintenance during root chilling is associated with the elevation and abiotic conditions of a species' native habitat. We also surveyed accessions from extreme elevations within chilling-sensitive *S. lycopersicum* var. *cerasiforme* and chilling-tolerant *S. habrochaites*. We assessed shoot turgor maintenance during root chilling in all available introgression lines (ILs) containing *S. habrochaites*, *S. lycopersicoides*, or *S. pennellii* alleles at the chromosome 9 region that is collinear to the QTL *stm9* region in *S. habrochaites* acc. LA1778 (Truco et al., 2000; Goodstal et al., 2005). Reciprocal grafted plants between the ILs and their respective cultivated parents were used to determine whether shoot turgor maintenance under root chilling is primarily a root- or shoot-determined trait.

MATERIALS AND METHODS

***Plant material*—**Accessions representing species within the section *Lycopersicon* of the genus *Solanum* and its nightshade allies *S. lycopersicoides* and *S. sitiens* were included in the initial survey of shoot wilting during root chilling (Table 1). These included salt-tolerant *S. cheesmaniae* and *S. galapagense,* drought-resistant *S. chilense* and *S. pennellii*, and cold-tolerant *S. lycopersicoides* and *S. habrochaites* (Patterson et al., 1978; Spooner et al., 2005). In a subsequent set of experiments, additional accessions of typically low elevation, nonstress tolerant *S. lycopersicum* var. *cerasiforme* and typically high elevation, chilling-tolerant *S. habrochaites* were also selected to represent the range of elevations for these tomato species (Table 2). Mean monthly climate data were extracted from Worldclim GIS maps (DIVA-GIS; http://www.worldclim.org) over the period from 1950 to 2000 (Hijmans et al., 2005) at the locations where the accessions were collected originally (Tables 1, 2).

A major QTL, *stm9*, from *S. habrochaites* acc. 1778 that controls shoot wilting under root chilling has been fine-mapped to a 2.7 cM interval (delineated by markers TG254 to CT143) (Truco et al., 2000; Goodstal et al., 2005). In addition, all four publicly available introgression lines (IL) that contain a single introgression from a wild species in the chromosome 9 region collinear to the 2.7 cM *stm9* region as determined by DNA markers in a genetic background of a *S. lycopersicum* cultivar were selected (Fig. 1). The ILs were: IL accession LA3957 with *S. habrochaites* acc. LA1777 alleles in a background of *S. lycopersicum* cv. E6203 (Monforte and Tanksley, 2000); IL acc. LA4080 with *S. pennellii* acc. LA0716 alleles in a background of *S. lycopersicum* cv. M82

(Eshed and Zamir, 1995); and IL acc. LA4268 and LA4269 with *S. lycopersicoides* acc. LA2951 alleles in a background of *S. lycopersicum* cv. VF36 (Rick and Yoder, 1988; Canady et al., 2005). The near-isogenic line (NIL) 03GH1322 (*S. habrochaites* acc. LA1778 alleles at the QTL *stm9* region in an otherwise *S. lycopersicum* cv. T5 background) and its parents were included as controls (Goodstal et al., 2005).

***Plant growth conditions*—**Seeds of the wild tomato species accessions, introgression lines, and cultivated varieties were obtained from the C. M. Rick Tomato Genetics Resource Center (http://tgrc.ucdavis.edu). In all experiments, wild tomato seeds were scarified in 2.6% aqueous NaClO for 30 min to overcome seed dormancy, if present. Seeds were germinated in flats containing a mix of vermiculite and perlite in the greenhouses at UC Davis. Greenhouse temperatures were 25–35°C day and 18–25°C night. The roots of seedlings, which were 8 to 15 d old depending on growth rate, were washed, and the plants transferred to aerated, temperature-controlled hydroponics growth tanks containing 50 mmol·m^{-3} NH_4NO_3 and the other elements maintained at 20% strength of a modified Hoagland solution (Epstein and Bloom, 2005).

Introgression lines for reciprocal grafting were grown in flats with standard potting mix for 20 d to use as shoot and root stocks. Seedling shoots were defoliated when they had two true leaves, cut below cotyledons, and splice grafted onto roots stocks; the grafts were protected by 2-cm-long sections of latex tubing. After new leaf growth was observed, roots were washed, and grafted plants were transferred to a hydroponic tank as described.

***Root chilling and shoot wilting scoring*—**In all experiments, after 7–10 d in a hydroponic tank at 20°C, plants were moved to a refrigerated hydroponics tank at 20°C and allowed to acclimate overnight. The following morning, under supplemental metal halide lighting to maintain the light level above 1000 μmol·m^{-2}·s^{-1} photosynthetic photon flux density at plant height, the tank temperature was lowered from 20°C to 6°C over 2 h and maintained at 6°C for an additional 2 h before scoring the shoot wilting phenotype of each plant. Shoot turgor maintenance under root chilling was scored on a scale of 0 to 3 (0 = completely turgid shoot, 1 = leaflet tips flaccid, 2 = more than 50% of leaves flaccid, 3 = completely wilted) as described in a previous study (Truco et al., 2000).

Solanum lycopersicum var. *cerasiforme* and *S. habrochaites* accessions were also measured for relative water content (RWC). Relative water content, a direct measure of shoot water stress, is the ratio of water volume in a leaf to the maximum water volume at full turgor. Relative water content was only compared within species as differing leaf anatomy between species may make RWC an inappropriate indicator for comparing water stress between species (Barrs, 1962). After scoring shoot wilting, leaves discs were cut, fresh mass (FW) was recorded, and the discs were immersed in distilled water in a sample cup at 5°C. The next day, the surface water was blotted-off discs, and the turgid mass (TW) recorded. Samples were then dried at 60°C to dryness (DW). Leaf relative water content was calculated using the formula: RWC (%) = (FW − DW)/(TW − DW) × 100 (Turner, 1981).

TABLE 1. Wild tomato accessions within the section *Lycopersicon* of the genus *Solanum* and its nightshade allies *S. lycopersicoides* and *S. sitiens* and their original collection site environmental characteristics. Accessions in bold are wild donor parents of introgression lines used in this study. All accessions were obtained from the Tomato Genetics Resource Center, U. C. Davis.

Accession	Taxon	Country	Elevation (m a.s.l.)	Mean temp (°C)	Mean low temp (°C)	Precipitation (mm/year)
LA1401	*S. galapagense*	Ecuador	5	23.0	19.5	744
LA1950	*S. pimpinellifolium*	Peru	20	19.4	16.1	0
LA0716	***S. pennellii***	**Peru**	**50**	**18.5**	**14.4**	**1**
LA1416	*S. pimpinellifolium*	Ecuador	500	24.9	20.9	2638
LA1421	*S. lycopersicum* var. *cerasiforme*	Ecuador	500	24.6	19.7	3380
LA0429	*S. cheesmaniae*	Ecuador	800	23.9	20.3	292
LA2680	*S. chmielewskii*	Peru	1650	17.8	9.8	1027
LA2951	***S. lycopersicoides***	**Peru**	**2200**	**11.2**	**3.4**	**57**
LA3657	*S. neorickii*	Peru	2430	15.0	6.8	746
LA3661	*S. chmielewskii*	Peru	2500	14.9	6.7	733
LA1927	*S. habrochaites*	Peru	2540	13.0	4.8	252
LA4110	*S. sitiens*	Chile	2600	11.2	3.7	17
LA1778	***S. habrochaites***	**Peru**	**2950**	**5.7**	**-1.5**	**641**
LA1777	***S. habrochaites***	**Peru**	**3150**	**5.7**	**-1.5**	**641**
LA2773	*S. chilense*	Chile	3400	7.4	−1.5	202

TABLE 2. Wild tomato accessions of typically low elevation, nonstress-tolerant *S. lycopersicum* var. *cerasiforme* and typically high elevation, chilling tolerant *S. habrochaites* and their original collection site environmental characteristics. All accessions were obtained from the Tomato Genetics Resource Center, U. C. Davis.

Accession	Country	Elevation (m a.s.l.)	Mean temp (°C)	Mean low temp (°C)	Precipitation (mm/year)
S. lycopersicum var. *cerasiforme*					
LA1953	Peru	20	19.7	16.3	18
LA1334	Peru	50	19.4	16.1	0
LA1421	Ecuador	500	24.5	19.7	3380
LA1231	Ecuador	550	23.1	18.2	4257
LA1228	Ecuador	1200	21.2	16.2	3005
LA1324	Peru	1200	20.7	12.8	1243
LA2633	Peru	1400	18.6	10.6	1417
LA2312	Peru	2100	15.6	10.1	1004
LA3652	Peru	2350	8.2	−0.1	769
LA2640	Peru	2400	16.5	8.4	754
S. habrochaites					
LA0407	Ecuador	40	25.3	21.0	811
LA2098	Ecuador	700	22.7	16.4	995
LA2650	Peru	800	22.5	16.0	622
LA2158	Peru	1500	17.1	10.6	847
LA2314	Peru	1650	15.0	8.9	889
LA2860	Ecuador	1700	19.2	12.9	1101
LA2119	Ecuador	2600	14.3	9.6	747
LA1778	Peru	2950	5.7	−1.5	641
LA1777	Peru	3150	5.7	−1.5	641

Experimental design and analyses—A total of 8 to 11 plants in a randomized complete block design for each accession, introgression line, and graft combination were scored for shoot wilting. Root chilling experiments of the initial survey of species within the section *Lycopersicon* of the genus *Solanum* and its allies *S. lycopersicoides* and *S. sitiens* were carried out on 13 May and 27 May 2011 and 28 October and 10 November 2011. Root chilling experiments of *S. lycopersicum* var. *cerasiforme* accessions were carried out on 15 and 27 October 2011 and 15 May 2012, and for *S. habrochaites* accessions on 21 November 2011 and 23 May and 13 June 2012. Root chilling experiments of chromosome 9 ILs and wild and cultivated parents were carried out on 17 March and 27 April 2011. Root chilling experiments of reciprocal grafts of introgression lines and cultivated parents were carried out between 21 June and 6 September 2012.

The effect of sampling date and genotype on shoot turgor maintenance under root chilling in accessions, introgression lines or graft combination (depending on the experiment) was tested for significance using analysis of variance (ANOVA) via the general linear model procedure (PROC GLM, SAS ver. 9.2, SAS Institute, Cary, North Carolina, USA). Means separation was determined using Tukey's tests ($P < 0.05$). Linear regressions were fitted using the program SigmaPlot 11 (Systat, Richmond, California, USA).

RESULTS

GIS maps affirmed that accessions in *Solanum* sect. *Lycopersicon* are native to a wide range of temperature and precipitation regimes (Table 1). For the wild accessions, the elevation and the coldest month's (June) mean daily minimum temperature of the collection site (Table 1) were significantly correlated with shoot turgor maintenance after 2 h of root chilling (Fig. 2A–C). Accessions originally collected from elevations greater than 2000 m a.s.l. face chilling temperatures (below 10°C) year round (Table 1) and experienced very little shoot wilting during root chilling (Fig. 2A). Accessions originally collected from elevations lower than 1000 m a.s.l. do not typically experience chilling temperatures in their native environments. These low elevation accessions became almost completely wilted after 2 h of root chilling (Fig. 2A). Although accessions were collected at sites where precipitation varied from 0 to over 3000 mm per year, there was no significant correlation between annual precipitation and wilting score during root chilling (Fig. 2D).

Among *S. lycopersicum* var. *cerasiforme* accessions (Table 2), shoot turgor maintenance during root chilling was not significantly correlated with collection site elevation or minimum temperature (Fig. 3A, B). There was, however, a significant correlation between annual precipitation and shoot turgor maintenance during root chilling (Fig. 3C). Leaf RWC, a direct measure of shoot water stress, was also significantly correlated with collection site annual precipitation (Fig. 3D).

Among *S. habrochaites* accessions (Table 2), shoot turgor maintenance during root chilling was not significantly correlated with site elevation, minimum temperature, or annual precipitation (Fig. 4). Mid-elevation accessions had a wide range of shoot turgor during root chilling from almost complete turgor maintenance to complete wilting and relative water content ranging from 77 to 89% (Fig. 4A, D).

Solanum habrochaites acc. LA1777, LA1778, and *S. lycopersicoides* acc. LA2951, which served as donor parents in the introgression lines (Fig. 1), originate from populations that grow at high elevation (>2000 m a.s.l.) (Table 1). In their native habitats, the wild populations represented by these accessions survive daily mean minimum temperatures well below the threshold for chilling damage of cultivated tomato (10°C) (Table 1). LA1777, LA1778, and LA2951 maintained shoot turgor during root chilling (Fig. 5). In contrast, *S. pennellii* acc. LA0716 is from exceedingly dry low elevations, but does not experience chilling in its native environment (Table 1). LA0716 did not maintain shoot turgor under root chilling (Fig. 5).
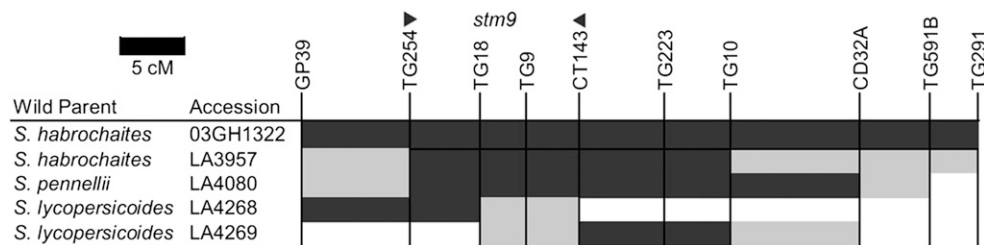

Fig. 1. Graphical scheme of genotypes of the chromosome 9 region in introgression lines obtained from C. M. Rick Tomato Genetics Resource Center (http://tgrc.ucdavis.edu) and for a chromosome 9 NIL (03GH1322) derived from *Solanum habrochaites* acc. LA1778 (Goodstal et al., 2005). Arrows indicate the major QTL, *stm9*. White bars indicate *S. lycopersicum* alleles, black bars indicate wild species alleles, and gray bars indicate regions of recombination breakpoints. Genetic distances (in cM) estimated from the *S. lycopersicum* × *S. pennellii* LA0716 mapping population (http://solgenomics.net).
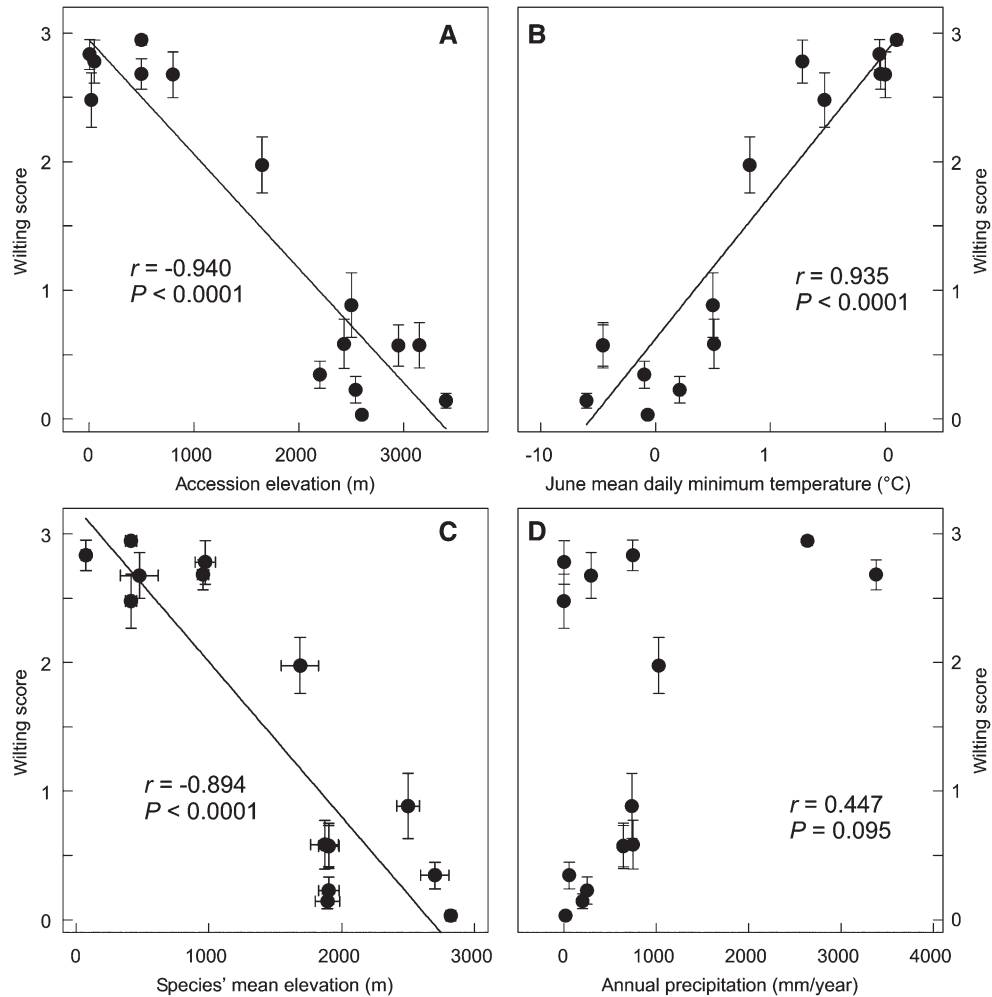
Fig. 2. Correlations between shoot wilting score (±SE) during root chilling and (A) native source population elevation, (B) winter minimum temperature (C) mean species elevation (±SE), and (D) annual precipitation for accessions in the genus *Solanum* sect. *Lycopersicon* and allies *S. lycopersicoides* and *S. sitiens* (see Table 1). Shoots were scored for wilting 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). Linear regressions fitted to data.

All of the *S. lycopersicum* cultivars, E6203, M82, and VF36, which served as the cultivated parent of the introgression lines, became fully flaccid during root chilling (Fig. 5). Introgression lines containing wild alleles at the collinear chromosome 9 region in the genetic background of *S. lycopersicum* had wilting scores intermediate of their wild and cultivated parents. Both *S. habrochaites* NIL 03GH1332 and IL acc. LA3957 wilted significantly less than their cultivated parents, T5 and E6203 respectively (Fig. 5). Both *S. lycopersicoides* ILs LA4268 and LA4269 wilted significantly less under root chilling than their cultivated parent VF36, although neither line contains the entirety of the chromosome 9 region collinear to QTL *stm9* from LA1778 (Figs. 1, 5). The *S. pennellii* IL LA4080 wilted under root chilling similar to its wild and cultivated parents (Fig. 5).

In all shoot to root grafts between introgression lines, only the root genotype had a significant effect on shoot turgor maintenance (Fig. 6). Grafts containing roots of LA1777, LA1778, or LA2951 maintained shoot turgor during root chilling regardless of shoot genotype (Fig. 6). Grafts containing only shoots of these nonwilting genotypes wilted during root chilling to the same extent as the *S. lycopersicum* cultivars (Fig. 6).

DISCUSSION

In our survey of shoot turgor maintenance during root chilling in *Solanum* sect. *Lycopersicon* species, accessions representing high elevation populations maintained shoot turgor during root chilling, suggesting that protecting shoots from water stress during root chilling influences fitness at high elevation habitats (Fig. 2A). Indeed, the propensity to close stomata to avoid water stress during root chilling is correlated with chilling tolerance in other species (Capell and Doerffling, 1993; Perez et al., 1997; Aroca et al., 2001, 2003). Likewise, shoot wilting during root chilling in accessions from low elevations suggests turgor maintenance during root chilling may be less adaptive (or less advantageous) in environments that do not experience chilling temperatures. Although stomatal closure reduces water loss, it can also limit photosynthesis and shoot growth (Kebede et al., 1994; Torrecillas et al., 1995; Martin et al., 1999).

The significant correlations between elevation or temperature of the accessions' original habitats and shoot turgor maintenance under root chilling primarily resulted from distinct high and low elevation groups (Fig. 2). Although relative water flow in detached
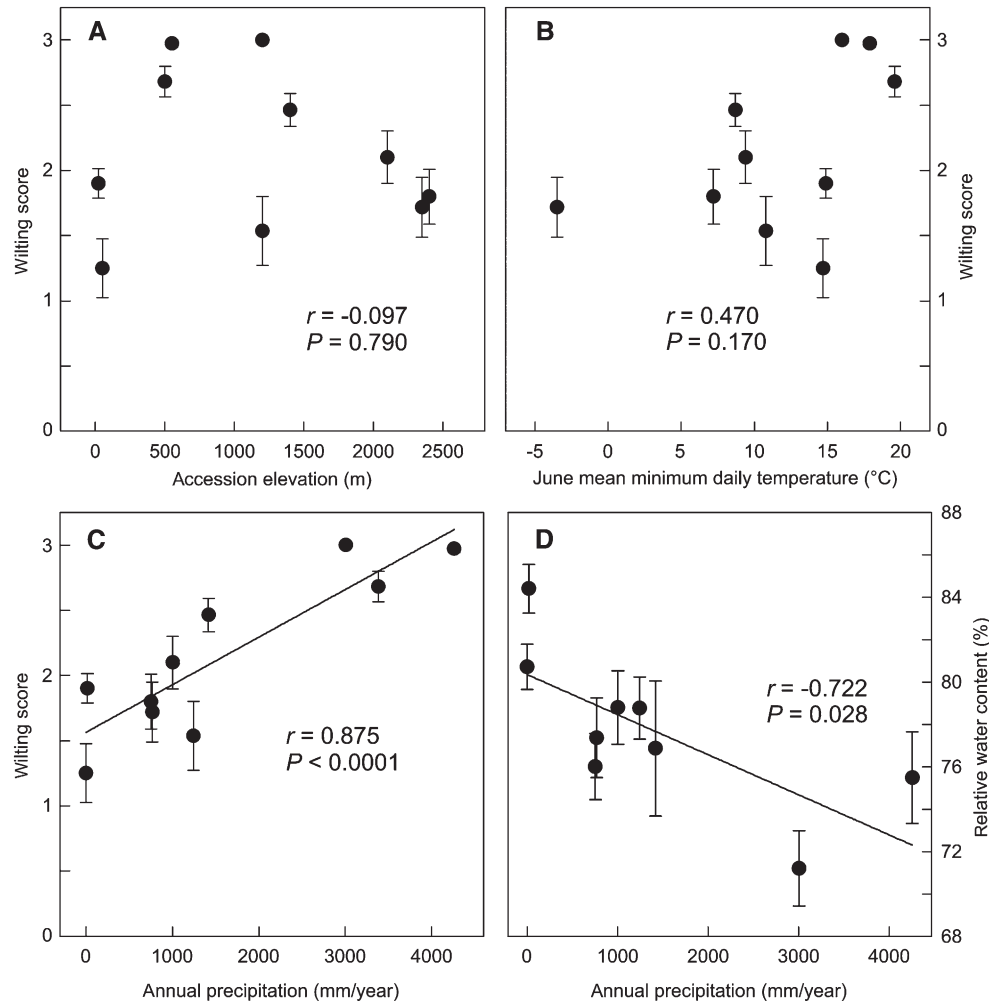
Fig. 3. Correlations between shoot wilting score (±SE) during root chilling and (A) native source population elevation, (B) winter minimum temperature, (C) annual precipitation, and (D) leaf relative water content and annual precipitation for accessions of *Solanum lycopersicum* var. *cerasiforme* (see Table 2). Shoots were scored for wilting 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). Linear regressions fitted to data.

tomato roots declined somewhat linearly with temperature, pressure compensation root chilling experiments in intact tomato plants showed that hydraulic conductance dropped precipitously below 8°C (Bloom et al., 2004). The presence of shoot turgor maintenance in high elevation species most likely represents adaptation to temperatures below 8°C. Indeed, high elevation accessions that maintained turgor (Fig. 2) are all from populations with mean winter low temperatures below 7°C (Table 1). The 8°C root temperature threshold also suggests that populations adapted to even lower temperatures would not necessarily have better turgor maintenance during root chilling. Variation in shoot turgor maintenance among high elevation populations may be observed at root temperatures lower than 6°C, but the plants were not subjected to such conditions in our study. The low elevation accessions that wilted (Fig. 2) all have mean winter low temperatures above 14°C (Table 1). This observation suggests that variation in shoot turgor maintenance at 6°C root temperature would not be advantageous unless it confers tolerance to other environmental stresses that the plants are likely to encounter.

In our study, we employed accession populations representative of species' mean elevation (Fig. 2C). Our GIS data showed

that accessions of *S. lycopersicum* var. *cerasiforme* and *S. habrochaites* are found over a wide range of elevations (Table 2). The *S. lycopersicum* var. *cerasiforme* accessions in our study represented populations from a wide range of elevations and had mean wilting scores greater than 1.2 during root chilling (Fig. 3). Shoot turgor maintenance was not correlated with mean elevation among accessions in *S. lycopersicum* var. *cerasiforme* (Fig. 3A). Interestingly, there was a significant negative correlation between annual precipitation and shoot turgor maintenance during root chilling (Fig. 3D), suggesting that some aspects of shoot turgor maintenance during root chilling may be advantageous for populations from habitats with low annual precipitation. Reduced stomatal conductance associated with water conservation in dry environments likely resulted in turgor maintenance under chilling-induced water stress (Bloom et al., 2004). Nonetheless, low elevation accessions of *S. pennellii*, *S. cheesmaniae*, *and S. pimpinellifolium* from exceedingly dry habitats did not maintain shoot turgor during root chilling (Fig. 2D). These tomato species use low stomatal frequency, decreased light absorption, and/or increased leaf thickness to limit shoot water loss, minimizing the importance of stomatal regulation
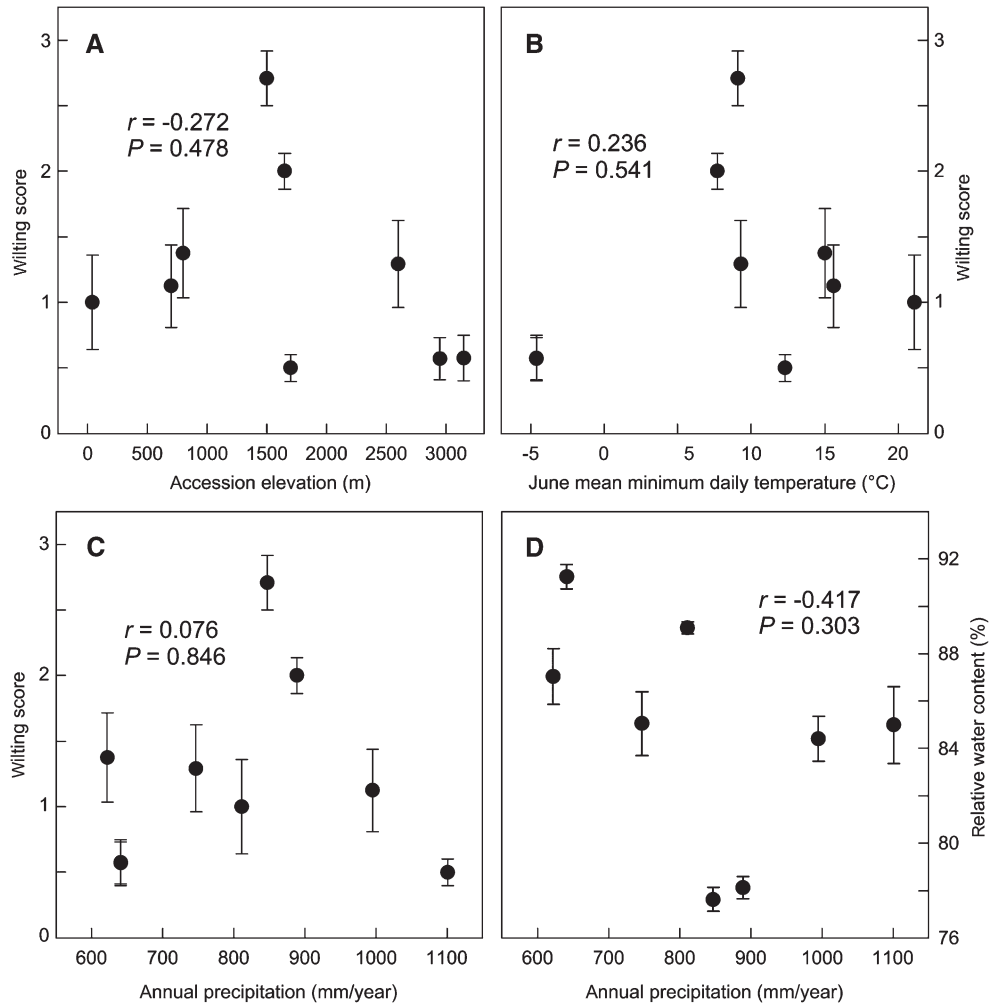
Fig. 4. Correlations between shoot wilting score (±SE) during root chilling and (A) native source population elevation, (B) winter minimum tempera-ture, (C) annual precipitation, and (D) leaf relative water content and annual precipitation for accessions of *Solanum habrochaites* (see Table 2). Shoots were scored for wilting on 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). Linear regressions fitted to data.

during water stress (Ramos et al., 1992; Kebede et al., 1994). The broad leaflets in *S. lycopersicum* var. *cerasiforme* acces-sions make them more susceptible to shoot water stress, which may increase the importance of stomatal regulation during water stress (Ehleringer et al., 1976; Easlon and Richards, 2009). Although some *S. lycopersicum* var. *cerasiforme* accessions maintained shoot turgor during root chilling (Fig. 3), all tested tomato varieties (T5, E6203, M82, and VF36) were chilling-sensitive and became completely wilted during root chilling (Fig. 5). Selection during domestication and breeding of crop species in high input agricultural systems for high yields can result in a loss of stress tolerance because such traits may limit photosynthesis and shoot growth and, thus, negatively influ-ence crop yield (Kebede et al., 1994; Torrecillas et al., 1995; Martin et al., 1999).

In *S. habrochaites*, accessions from lowest and highest ele-vations maintained turgor during wilting similarly to accessions observed in the *Solanum* sect. *Lycopersicon* survey (Fig. 2), but two accessions from mid-elevations did not (Fig. 4A). Shoot turgor maintenance was not correlated with mean elevation among *S. habrochaites* accessions from a wide range of native

habitat elevations (Fig. 4A). Whereas crop species may not sur-vive in stressful environments, stress adapted species can grow in nonstressful environments, albeit sometimes at a slower rate. The observation that some low and mid-elevation accessions of *S. habrochaites* retain the ability to maintain shoot turgor under root chilling does not imply that this trait is irrelevant to sur-vival at high elevation. In future investigations, the markers defining the chromosome 9 region collinear to the *stm9* QTL could be used to genotype the mid-elevation *S. habrochaites* accessions to determine whether interspecific hybridizations with low-elevation species resulted in a transfer of alleles that led to the loss of turgor maintenance during root chilling. Annual precipitation was not correlated with shoot turgor main-tenance during root chilling in the *S. habrochaites* accessions evaluated in this study, but *S. habrochaites* in this study were not from extremely arid environments, limiting the ability to detect such correlations.

Accessions of *S. lycopersicoides* and *S. habrochaites*, which experience chilling temperatures in their native habitats, main-tained shoot turgor during root chilling (Table 1, Fig. 5). Like-wise, ILs containing the *S. habrochaites* or *S. lycopersicoides*
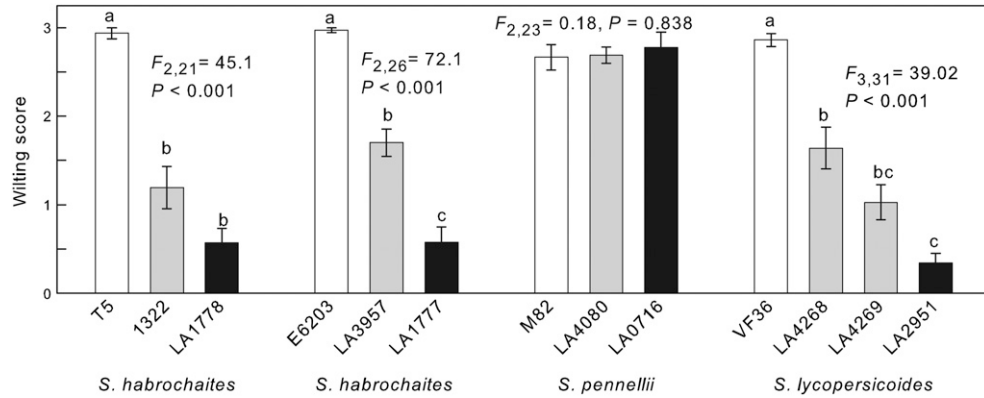
Fig. 5.   Shoot wilting scores (±SE) during root chilling in tomato chromosome 9 introgression lines or NIL 03GH1322 (gray) and their wild accession (black) and cultivated variety (white) parents. A score of 3 indicates that the shoots were fully flaccid; a score of 0 indicates that they were fully turgid. Letters indicate significant differences within groups according to Tukey's tests ($P < 0.05$).

alleles collinear to *stm9* maintained a higher degree of shoot turgor with root chilling than their respective cultivated parent did (Fig. 5). These results suggest adaptation to chilling temperatures in wild tomatoes may have involved orthologous gene(s) in the collinear *stm9* region for regulating stomatal sensitivity to root chilling. Because both *S. lycopersicoides* ILs LA4268 and LA4269 maintained turgor during root chilling, gene(s) conferring shoot turgor maintenance may be present in the overlapping chromosomal introgressions in these ILs. Alternatively, ILs LA4268 and LA4269 may contain other gene(s)

conferring shoot turgor maintenance in the unshared, adjacent chromosome 9 regions. Additional fine mapping would clarify the location of gene(s) conferring shoot turgor maintenance during root chilling in ILs LA4268 and LA4269.

Solanum pennellii is native to exceedingly dry environments, where rapid stomatal response to water deficit could be beneficial (Rick, 1973, 1979; Rick et al., 1978; Smith and Peralta, 2002; Young et al., 2002; Nakazato et al., 2008; Easlon and Richards, 2009; Nakazato et al., 2010). Nonetheless, both *S. pennellii* acc. LA0716 and the IL LA4080 containing the *S. pennellii* allele at
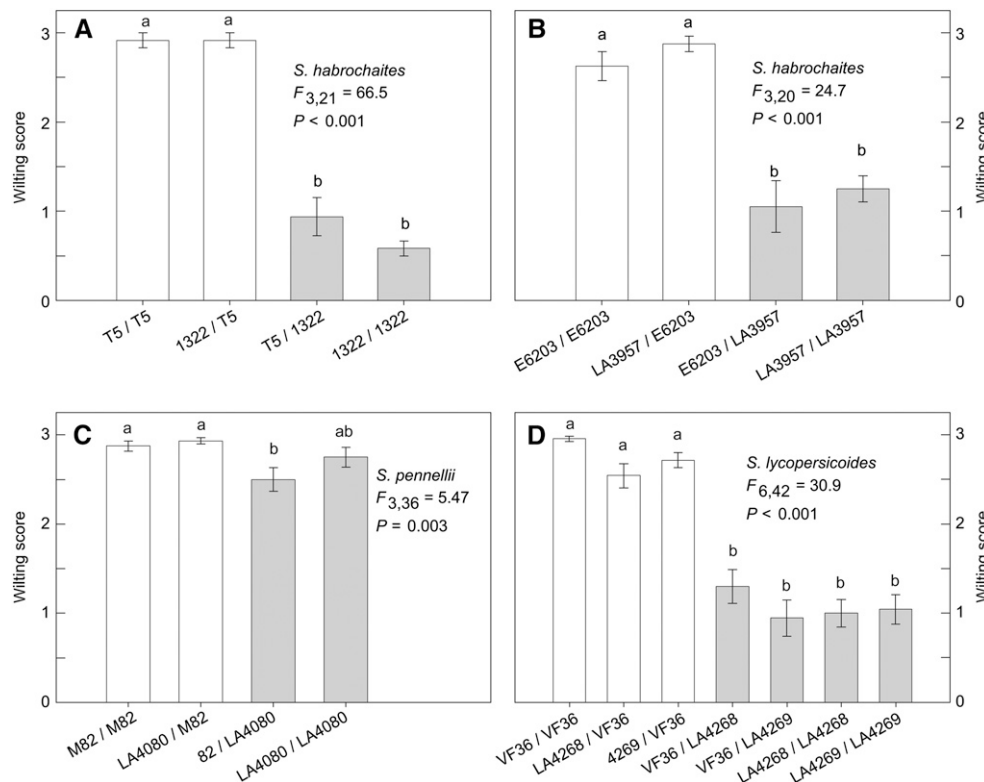

Fig. 6.   Shoot wilting scores (±SE) during root chilling in grafts of tomato chromosome 9 introgression lines or NIL 03GH1322 and cultivated variety parents (denoted as shoot genotype / root genotype). Gray bars indicate roots have wild alleles at regions collinear to *stm9*. A score of 3 indicates that the shoots were fully flaccid; a score of 0 indicates that they were fully turgid. Letters indicate significant differences according to Tukey's tests ($P < 0.05$).

the collinear chromosome 9 region became fully flaccid with root chilling (Fig. 5). The failure of the *S. pennellii* acc. LA0716 and the IL LA4080 to exhibit stomatal sensitivity to root chilling suggests mechanisms conferring drought tolerance in *S. pennellii* differ from mechanisms conferring turgor maintenance during root chilling in *S. lycopersicoides* and *S. habrochaites*. *Solanum lycopersicum* var. *cerasiforme* accessions from exceedingly dry environments lack other leaf adaptations (i.e., increased leaf thickness and lower stomatal frequency) to drought stress found in *S. pennellii* (Kebede et al., 1994). Drought stress in *S. lycopersicum* var. *cerasiforme* accessions lacking these adaptations may more rapidly affect leaf water potential. Indeed, the plant response to root chilling has more in common with the response to rapid onset water stress that is distinct from the response to chronic drought that progresses more gradually (Markhart et al., 1979; Bagnall et al., 1983; Fennell and Markhart, 1998; Aroca et al., 2001; Bloom et al., 2004).

Reciprocal shoot–root-grafted young plants of introgression lines and their cultivated parents showed that turgor maintenance during root chilling conferred by chromosomal regions collinear to the major effect QTL *stm9* are root-based. All grafted plants containing chilling-tolerant IL roots maintained shoot turgor during root chilling regardless of shoot genotype (Fig. 6). Roots of *S. pennellii* IL had no effect or a very small effect on shoot turgor maintenance depending on shoot genotype (Fig. 6C). That shoot genotype had little effect suggests that leaf characteristics may not have a significant effect on shoot turgor maintenance during root chilling. Our prior study on root-chilling response of shoot–root grafts between *S. lycopersicum* (formerly *L. esculentum*) cv. T5 and the progeny of this species and *S. habrochaites* acc. LA1778 suggested that both shoot and root play roles in the shoot response to root chilling (Bloom et al., 2004). The difference in results most likely derives from *S. habrochaites* alleles at genes on other chromosomes in the interspecific F$_1$ hybrid that are not present in the chromosome 9 ILs examined here.

Resistance to chilling-induced water stress found in wild species from high elevations suggests root chilling has been an important driver of adaptive diversification in wild tomatoes. Resistance to root chilling appears to involve mechanisms both independent from and shared with drought resistance. Although accessions of *S. lycopersicum* var. *cerasiforme* representing extremely dry native habitats maintained some shoot turgor during root chilling, drought-adapted *S. pennellii* completely wilted during root chilling (Figs. 1, 3, 5). Adaptations to soil drought in *S. lycopersicum* var. *cerasiforme* may involve stomatal responses to rapid onset water stress that are not present in *S. pennellii*. Our study also identified root-based resistance to chilling-induced water stress in the region collinear to the QTL *stm9* in high elevation populations of *S. habrochaites* and *S. lycopersicoides*. Root-based resistance to chilling-induced water stress may provide alternatives to shoot mechanisms for improving crop responses to drought.

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