Plant, Cell and Environment (2004) **27**, 971–979

Water relations under root chilling in a sensitive and tolerant tomato species

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ABSTRACT

The shoots of cultivated tomato (*Lycopersicon esculentum* cv. T5) wilt if their roots are exposed to chilling temperatures of around 5 °C. Under the same treatment, a chilling-tolerant congener (*Lycopersicon hirsutum* LA 1778) maintains shoot turgor. To determine the physiological basis of this differential response, the effect of chilling on both excised roots and roots of intact plants in pressure chambers were investigated. In excised roots and intact plants, root hydraulic conductance declined with temperature to nearly twice the extent expected from the temperature dependence of the viscosity of water, but the response was similar in both species. The species differed markedly, however, in stomatal behaviour: in *L. hirsutum*, stomatal conductance declined as root temperatures were lowered, whereas the stomata of *L. esculentum* remained open until the roots reached 5 °C, and the plants became flaccid and suffered damage. Grafted plants with the shoots of one genotype and roots of another indicated that the differential stomatal behaviour during root chilling has distinct shoot and root components.

Key-words : chilling injury; chilling tolerance; hydraulic conductance; stomatal conductance.

INTRODUCTION

The cultivated tomato, *Lycopersicon esculentum* Mill., is a classic example of a chilling-sensitive plant. Temperatures below 10 °C severely inhibit tomato growth and development at all life stages, and those below 6 °C inflict significant injury (Geisenberg & Stewart 1986). By contrast, an interfertile wild species, *Lycopersicon hirsutum* , grows in the Peruvian Andes at altitudes up to 3300 m and thrives at chilling temperatures that are detrimental to *L. esculentum* (Patterson, Paull & Smillie 1978; Dalziel & Breidenbach 1982; Vallejos & Tanksley 1983; Wolf *et al*. 1986; Yakir, Rudich & Bravdo 1986; Vallejos & Pearcy 1987; Jung, Steffen & Lee 1998; Venema *et al*. 1999). The physiological basis for this differential chilling sensitivity remains uncertain, although several hypotheses have been put forward (Bowers 1994; Guy 1994; Li 1994; Kaye & Guy 1995; Nishida & Murata 1996; Pearce 1999).

One hypothesis is that chilling causes transitions in membrane lipids from a fluid phase to a gel phase that impairs membrane permeability (Lyons & Raison 1970); in the specific case of *L. esculentum* and *L. hirsutum* , however, phase transitions in leaf membranes occur at similar temperatures (Dalziel & Breidenbach 1982; Marangoni & Stanley 1989; Raison & Brown 1989). Another possibility is that chilling might inhibit energy metabolism or produce accumulations of deleterious by-products (Guy 1994); in leaves of *L. esculentum* , such changes occur only after several hours of chilling at high light and have little immediate or lasting effect upon photosynthesis (Martin & Ort 1985; Yakir *et al*. 1986; Vallejos & Pearcy 1987; Sassenrath, Ort & Portis 1990; Venema, Villerius & van Hasselt 2000).

Chilling also damages roots. As far back as 1727, Stephen Hales conducted experiments showing that seedlings wilt in cold soils because chilling impedes root water absorption (Hales 1727). Chilling injury in several species may result from water loss through open stomata at a time when root hydraulic conductance is low (Wilson 1976; Markhart *et al.* 1979; Bagnall, Wolfe & King 1983; Fennell & Markhart 1998; Aroca *et al*. 2001). After longer exposures to chilling, plants may recover from wilting through stomatal closure, restoration of root hydraulic conductance to higher values, and enhanced root ion absorption and carbohydrate accumulation (Markhart *et al*. 1979; Bagnall *et al*. 1983; Ali *et al*. 1996; Fennell & Markhart 1998; Vernieri *et al*. 2001).

Shoots of the chilling-sensitive *L. esculentum* wilt if their roots are exposed to chilling temperatures of around 5 °C, whereas shoots of the chilling-tolerant *L. hirsutum* maintain turgor under the same treatment. Backcross progeny of these species vary widely in the degree to which their shoots maintain turgor under root chilling (Truco *et al*. 2000). In the following study, we examined the water relations of *L. esculentum* , *L. hirsutum* , and grafted plants consisting of shoots and roots from different genotypes to determine the relative contributions of shoot and root characteristics to the chilling sensitivity of tomato.

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MATERIALS AND METHODS

Plant material and growth conditions

Lycopersicon esculentum cv. T5 is a chilling-sensitive, highly self-pollinated, fresh market tomato cultivar. *Lycopersicon hirsutum* LA 1778 is an outcrossing accession that was collected at 3200 m elevation in the Peruvian Andes. Seeds of both species were obtained from the C. M. Rick Tomato Genetic Resource Center at UC Davis (http://tgrc.ucdavis.edu). They were surface-sterilized (15 min in 1.3% NaClO for *L. esculentum* and 30 min in 2.6% NaClO for *L. hirsutum*).

For the material used in most of the experiments, seeds were germinated on several layers of cheesecloth placed over the surface of a 19 dm^3 plastic tub filled with 1.0 mol m^{-3} $CaSO_4$ and 50 mmol m^{-3} NH_4NO_3. Vigorous aeration kept the cheesecloth moist. Once the roots reached the nutrient solution, it was changed to a more complete one containing 50 mmol m^{-3} NH_4NO_3 and the other elements at 20% the concentrations of a modified Hoagland solution (Epstein & Bloom 2004). The plants were placed in a controlled environment chamber (Conviron, Winnipeg, Canada) set at 25 °C day/18 °C night with a 16-h photoperiod. The photosynthetic flux density (PFD) was 500–600 μmol m^{-2} s^{-1} at plant height. The nutrient solution was replenished every 7 d. Experiments were conducted on 17- to 19-day-old-plants that had two fully expanded leaves. The night before an experiment, plants were brought into the laboratory and allowed 12 h or longer in the dark and 3 h at 700 μmol m^{-2} s^{-1} PFD to recover from transplant shock.

For the material used for measurements of hydraulic conductance of whole plants, seeds were germinated in well-fertilized, friable potting mix in special pots (see below). The pots were placed in a greenhouse with a 25 °C, 12 h day and a 20 °C, 12 h night, and were brought into the laboratory when the plants were 14–17 d old.

Water relation measurements

In the hydraulic conductance measurements of excised roots, a long primary root was cut below the hypocotyl, an o-ring was fitted around the cut end, and the root sealed into a pressure chamber (Fig. 1). Flexible tubing was pressed over the stele of the cut end of the root; the other end of this tubing added fluid expressed from the xylem into a 10-mL graduated cylinder sitting on a sensitive balance (Sartorius BP211D; ± 0.00001 g; Sartorius, Goettingen, Sweden). The solution in the cylinder was covered by a thin layer of olive oil. Compressed air flowed into the pressure chamber to aerate the root as well as to raise the pressure; a needle valve controlled the rate of flow and thereby the pressure was monitored with a pressure transducer (Omega PX236-GV100; Omega Engineering, Stamford, CT, USA). The pressure chamber had a heat-exchange coil through which water and antifreeze flowed from a refrigerated water bath (Forma 2006; Thermo Forma, Marietta, OH, USA) to control temperature. A data acquisition system monitored changes in root temperature, chamber pressure, and the mass of the expressed xylem fluid as described in Zwieniecki, Thompson & Holbrook (2002). Roots of five plants of each species were examined.

A stainless steel heat-exchange coil was placed in the 19 dm^{-3} tub containing six to nine plants per species and chilled solution circulated through the coil. We monitored (a) root temperature; (b) stomatal conductance with a Licor LI-1600 steady-state porometer (Li-Cor Inc., Lincoln, NE, USA); (c) shoot water potential with Soil Moisture Equipment 3000 pressure chamber (Santa Barbara, CA, USA); and (d) leaf water potential and osmotic potential (before and after freezing in liquid N_2) with a Wescor 5100 thermocouple psychrometer (Logan, UT, USA).

The hydraulic conductance of whole plants was measured using techniques described by Stirzaker & Passioura (1996) and Passioura & Munns (2000). In brief, plants were grown in special pots that could be enclosed in a pressure chamber with a silicon rubber pressure seal at the junction between root and shoot (Fig. 2). A fine nylon tube was inserted into a cut in the xylem at the base of the stem and connected to a water-filled capillary tube. As the nylon tube was hydrophilic, it maintained hydraulic continuity between the xylem sap and the capillary tube provided that the xylem sap was close to atmospheric pressure. If the pressure in the chamber was too high, sap bled out of the xylem and the meniscus in the capillary tube rose and cut the infra-red beam; if the pressure was too low, the sap

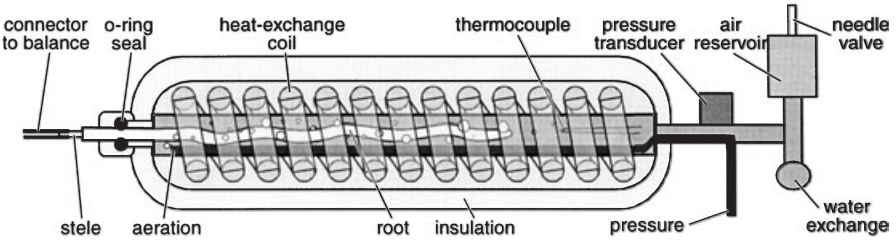

Figure 1. An apparatus to monitor hydraulic conductance through an excised root. An o-ring compressed by a screw cap sealed the root into a pressure chamber. Tubing directed the xylem fluid expressed from the root onto an electronic balance (not shown) to monitor the rate of water flow (± 0.01 mg). Compressed air bubbled through the nutrient solution in the chamber to provide oxygen to the root as well as to pressurize the chamber. A refrigerated water bath (not shown) pumped fluid through a heat exchange coil in the chamber to control temperature.

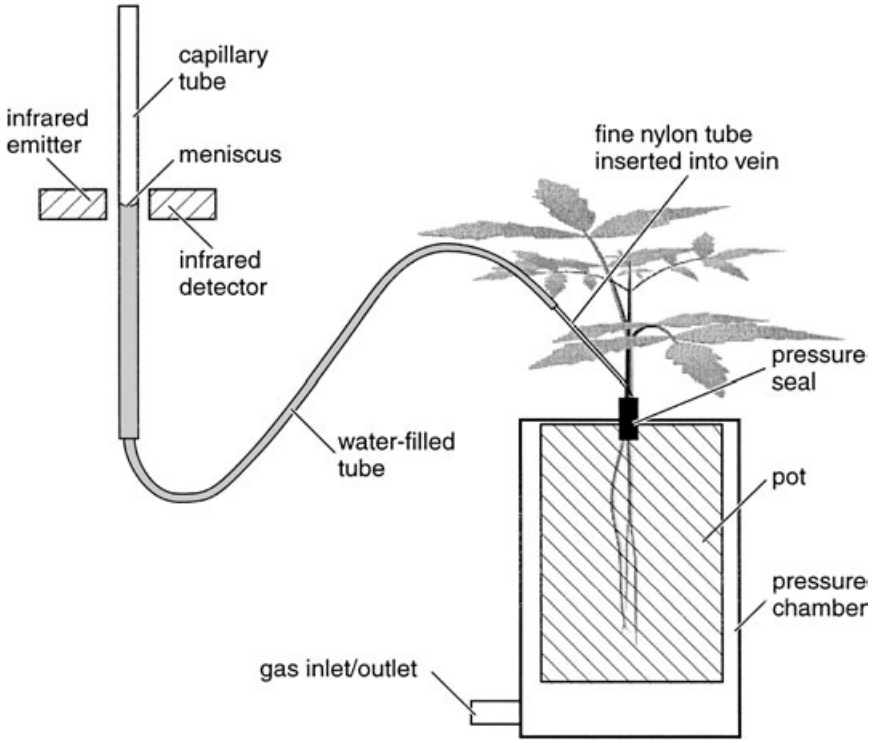

Figure 2. Apparatus to monitor hydraulic conductance of the root system of an intact plant. A silicon gasket sealed the roots into a pressure chamber. A fine nylon tube was inserted into a cut in the xylem at the base of the stem and was connected to a water-filled capillary tube. Because the nylon tube was hydrophilic, it maintained hydraulic continuity between the xylem sap and the capillary tube provided that the xylem sap was close to atmospheric pressure. If the pressure in the chamber was too high, sap bled out of the xylem and the meniscus in the capillary tube rose and cut the infrared beam; if the pressure was too low, the sap retreated into the xylem thereby lowering the meniscus and exposing the infrared beam. A control system (not shown) adjusted the pressure in the chamber to maintain the meniscus at a constant level.

retreated into the xylem thereby lowering the meniscus and exposing the infrared beam. A control system adjusted the pressure in the chamber to maintain the meniscus at a constant level. This applied pressure (the 'balancing pressure') was equal to the pressure drop across the root system, between the soil solution and the xylem at the base of the shoot.

The shoot was enclosed in a temperature-controlled cuvette. The glass walls of the cuvette were 150 mm diameter by 250 mm long and mounted vertically on a polyvinyl chloride (PVC) base containing a central, 50-mm diameter hole through which the shoot, thermocouple wires, tubing for the meniscus sensor, and air entered the cuvette. The PVC base clamped onto the pressure chamber, with an intervening rubber gasket that prevented loss of air from the cuvette, while allowing the wires and tubes to enter. A PVC plate that formed the top of the cuvette contained a fan, which rotated just fast enough to induce gentle leaf flutter, and an air outlet from which air was sent to the humidity sensor. The humidity sensor was a chilled mirror hygrometer (General Eastern DEW-10–0D1; General Eastern Instruments, Wilmington, MA, USA). The flow rate of the air through the cuvette was measured with a mass flowmeter (Hastings EALL-5KP or EALL-50KP; Teledyne Hastings Instruments, Hampton, VA, USA) and was adjusted within the range of 2 to 8 L min^{-1} according to transpiration rate to keep an easily measurable difference in dew point between the ingoing and outgoing air and a difference in vapour pressure of about 0.5 kPa.

The transpiration rate of the whole shoot was varied by changing the humidity of the air entering the cuvette or the light intensity, and was measured as the product of the flow rate of air through the cuvette and the difference in humidity between the ingoing and outgoing air. The slope of transpiration rate as a function of balancing pressure provided an estimate of hydraulic conductance (Steudle 1992). Three plants of each species having similar leaf areas were monitored.

We calculated the hydraulic conductance values at 20 and 10 °C and Arrhenius activation energy between these temperatures. Plants were subjected to temperatures as low as 5 °C, but balancing pressures became unstable at temperatures lower than 10 °C and thus calculation of hydraulic conductance became unreliable. Statistical differences between the species were assessed with the general linear model in SAS (PROC GLM; SAS Institute, Cary, NC, USA). Effect of species was considered significant when $P < 0.05$.

Grafted plants and chilling response

The two parents, *Lycopersicon esculentum* cv. T5 and *L. hirsutum* LA 1778, proved to be incompatible for grafting: grafted plants with roots from one species and the shoot from the other would thrive for about 10 d and then die suddenly. Fortunately, we were able to obtain successful shoot/root grafts between *L. esculentum* cv. T5 and the progeny of this species and *L. hirsutum* LA 1778. 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.

Table 1. The response of hydraulic conductance to root temperatures and Arrhenius activation energy in chilling-sensitive *L. esculentum* cv. T5 (E) and chilling tolerant *L. hirsutum* LA1778 (H) as measured in excised roots or in intact plants

	Excised roots			Intact plants		
	Hydraulic conductance (mg root^{-1} s^{-1} kPa^{-1})			Hydraulic conductance (mg m^{-1} s^{-1} kPa^{-1})		
Genotype	20 °C	10 °C	Activation energy (kcal mol^{-1})	20 °C	10 °C	Activation energy (kcal mol^{-1})
E	97 ± 37	58 ± 22	9.0	0.31 ± 0.03	0.18 ± 0.03	9.5
H	76 ± 18	44 ± 8	9.4	0.10 ± 0.01	0.06 ± 0.01	8.4

The hydraulic conductances from the pressure pot are normalized per leaf area. Values shown for the hydraulic conductances are the means ± SE ($n = 5$ plants for excised roots and $n = 3$ for intact plants) and for the activation energies are the means. For reference, the activation energy for water traversing a water-filled pore is about 4 kcal mol^{-1}

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* . We have found previously that alleles from *L. hirsutum* LA 1778 at a quantitative trait locus (QTL) on chromosome 9 are strongly associated with shoot turgor maintenance under chilling (Truco *et al*. 2000). We refer to this QTL as *stm9* in Table 2. A single BC_1 ($BC_1$33), heterozygous for the *L. hirsutum* alleles at the QTL on chromosome 9, served as the pollen donor in the second backcross to T5. The BC_2 plants 4428 and 4507 are heterozygous at this same QTL. In summary, the genotypes used to make grafts were: T5, $BC_1$33, BC_2-4428, and BC_2-4507.

The most successful grafting technique was to have a plant of one genotype potted in a standard potting mix serve as the root stock. Shoot stock about 25 mm long was cut from a plant of the same or another genotype, and all but the newest leaves were removed. Graft cuts were made in node areas of both the root and shoot stock. Cuts were a deep V in the root stock and a corresponding wedge in the shoot. The union was wrapped with pre-stretched Parafilm (Pechiney Plastic Packaging, Chicago, IL, USA), and the shoot covered with a clear, ventilated plastic bag for a week to reduce water loss. All grafted plants were grown in a greenhouse in Davis, CA. Once a grafted plant showed signs of new growth, the root stock was cut 50 mm below the graft area, dipped in 0.20% 1 Naphthaleneacetamide and 4.04% Thiram (synthetic auxin and fungicide, Rootone®; Gulfstream Home & Garden, Lexington, KY), placed in a vermiculite/perlite mix, and the Parafilm removed. Grafted plants were transferred to 19 dm^{-3} plastic tubs when their roots were 25 mm long, and were grown in solution culture as described above.

When the grafted plants had developed two new fully expanded leaves, we evaluated the chilling sensitivity of shoot wilting. The roots were chilled to 4 °C whereas the shoots remained at room temperature of 20 °C. Shoot wilting was scored visually on a scale from 0 (fully turgid) to 3 (fully flaccid) after 2 h at 4 °C. This measure has proved highly correlated with the ability of tomato genotypes to resume rapid growth after a chilling episode (Bloom, unpublished results) and was used to identify QTLs for shoot turgor maintenance (Truco *et al*. 2000). Differences among the grafts for shoot wilting were analysed for mean separation via a Bonferroni (Dunn) *t*-test (SAS Institute Procedure GLM). Because the differences in the wilting scores among BC_1-33, BC_2-4428, and BC_2-4507 were insignificant (data not shown), the data for these genotypes were pooled and designated as the non-wilting phenotype (*nw*) and the data for T5 was designated as the wilting phenotype (*w*).

RESULTS

Water flow through the roots of *L. esculentum* cv. T5 and *L. hirsutum* LA 1778 declined to a similar extent with a decrease in temperature. In both species, the Arrhenius activation energy (E_a) was about 9 kcal mol^{-1} (Table 1, Fig. 3). Leaf water potentials remained relatively constant in *L. hirsutum* during the chilling episode, whereas they

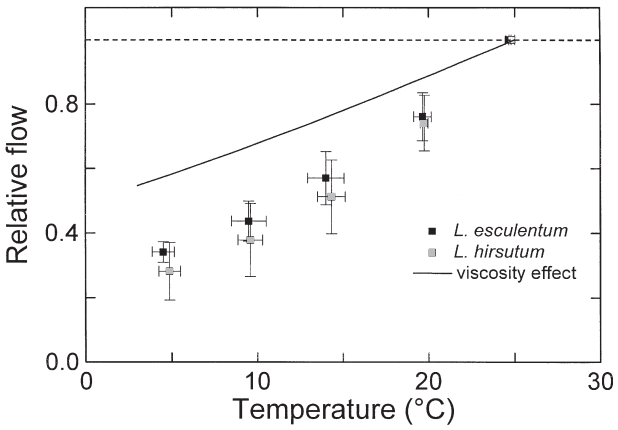

Figure 3. Relative water flow through excised roots of the chilling-sensitive *Lycopersicon esculentum* cv. T5 (dark squares) and the chilling-tolerant *L. hirsutum* LA1778 (light squares) as a function of root temperature, where the flow at 25 °C is taken as the reference point. Shown are the mean ± SE ($n = 5$ plants) with small errors bars incorporated into the symbols. The solid line indicates the theoretical response of water flow through a water-filled pore resulting from changes in the viscosity of water.

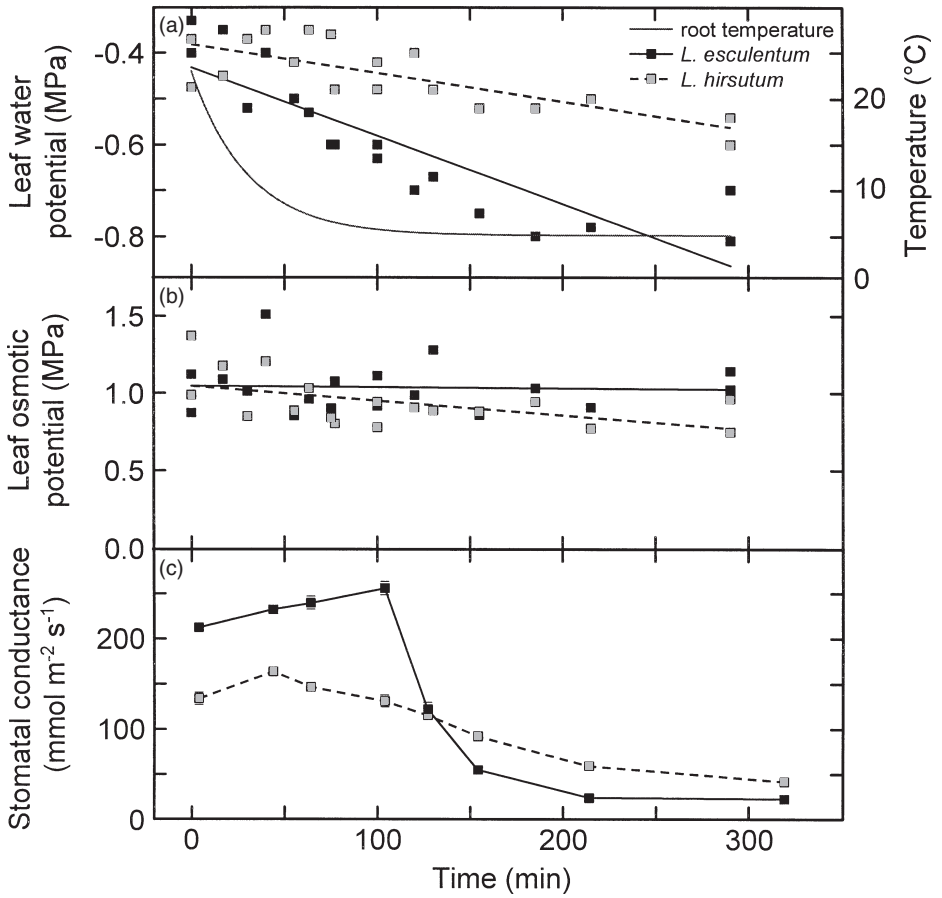

Figure 4. Changes in the water relations of *Lycopersicon esculentum* cv. T5 (*E*) and *L. hirsutum* LA1778 (*H*) as the root temperature declined from 25 to 5 °C. (a) The temperature response of leaf water potential. The merged data for three plants of each genotype are shown, *E* (dark squares) and *H* (light squares), and a linear regression of these data. (b) The temperature response of leaf osmotic potential. Shown are the merged data for three plants of each genotype are shown, *E* and *H*, and a linear regression of these data. (c) The temperature response of stomatal conductance. The mean ± SE ($n = 5$ plants) are shown with small errors bars incorporated into the symbols.

dropped steadily in *L. esculentum* (Fig. 4a). Leaf osmotic potential declined slightly with temperature in *L. hirsutum*, but not in *L. esculentum* (Fig. 4b). The two species differed markedly in their stomatal behaviour (Fig. 4c). Stomatal conductances at moderate temperatures were smaller in *L. hirsutum* than in *L. esculentum* and declined gradually as root temperatures were lowered, whereas the stomata of *L. esculentum* remained open until the roots reached 5 °C and the plants became flaccid.

In the pressure pot experiments on whole plants, *L. esculentum* had about three times the hydraulic conductance of *L. hirsutum* (Table 1), but both species showed similar relative declines in hydraulic conductance at low temperatures, with Arrhenius activation energies of about 9 kcal mol^{-1} (Table 1). When root temperatures dipped below 8 °C, the root pressure–water potential–transpiration relationships in both genotypes became unstable: the balancing pressure required to maintain shoot water status continued to rise for several hours without reaching an equilibrium (Fig. 4). Nonetheless, hydraulic conductance recovered within an hour upon re-warming. Stomatal conductances were similar in both species at 20 °C, and they declined more rapidly with temperature in *L. hirsutum* than in *L. esculentum* (Fig. 5). Note that in these plants leaf water potential was maintained high at all times because the root chamber was pressurized to maintain the xylem sap at the base of the shoot at atmospheric pressure.

Grafting *per se* had little effect on the wilting response: plants in which the shoots of one phenotype were grafted to roots of the same phenotype did not differ in their wilting response from their respective ungrafted controls (*w/w* versus *w* and *nw/nw* versus *nw*, using the nomenclature *shoot/root*; Table 2). Comparing reciprocal grafts between differing phenotypes with the self-grafts, namely, *w/w* versus *w/nw* versus *nw/w* versus *nw/nw*, the *w/w* wilted the most, the *nw/nw* wilted least, and the plants composed of different genotypes and their accompanying phenotypes for the shoot and root showed similar intermediate responses (Table 2).

DISCUSSION

Two independent methods showed that water movement through the roots was equally temperature-dependent in a chilling-sensitive (*L. esculentum* cv. T5) and a chilling-tolerant (*L. hirsutum* LA1778) species (Fig. 3, Table 1). Water, because its viscosity decreases slightly with temperature, traverses a water-filled pore with an activation energy (E_a) of about 4 kcal mol^{-1} (Finkelstein 1987). The water movement through the roots, however, had E_a values of around 9 in both species, indicating that it involves more than water flowing through pipes. For comparison, E_a values for water transport across a lipid bilayer via solubility-diffusion are typically around 10 kcal mol^{-1}, whereas those

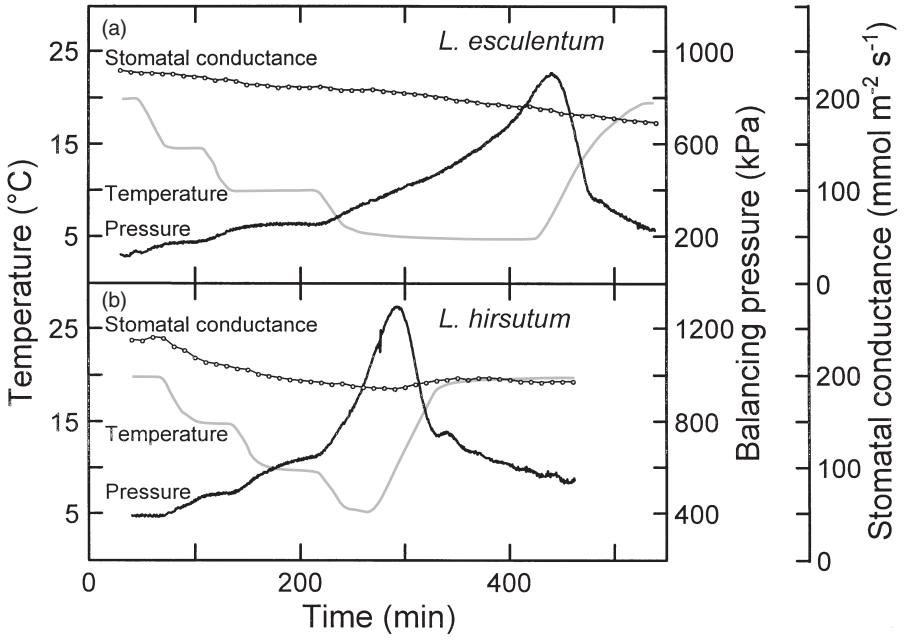

Figure 5. Typical traces for the pressure in the root chamber (dark line) necessary to hold the xylem sap at the base of the stem at atmospheric pressure (the 'balancing' pressure) and stomatal conductance (line with circles) at different root temperatures (light line). The chilling-sensitive *L. esculentum* cv. T5 (a) and chilling-tolerant *L. hirsutum* LA1778 (b) showed similar responses. At moderate temperatures, the pressure reached a steady level within an hour after root temperatures changed. If root temperatures dropped below 8 °C, however, the pressure continued to climb for several hours until root temperatures returned to above 8 °C. Stomatal conductance quickly showed a small decline with temperature in *L. hirsutum*, but slowly and steadily declined in *L. esculentum*.

for movement through water channels such as aquaporins are below 6 kcal mol^{-1} (Elmoazzen, Elliott & McGann 2002). Therefore, the E_a values observed here (approximately 9) suggest that a similar membrane barrier limits or controls water movement through the roots of both species.

In intact plants of both species, hydraulic conductance became unstable when root temperatures dipped below 8 °C, as evidenced by the accelerating rise in balancing pressure (Fig. 5). Hydraulic conductance recovered, however, within an hour upon re-warming. We, as of yet, have no straightforward explanation for this phenomenon.

Table 2. Wilting of shoots when roots were exposed to 4 °C for 2 h in various tomato phenotypes

Phenotype shoot/root	*n*	Wilting score	Bonferroni groups
w	42	2.73	a
w/w	22	2.38	a
nw/w	26	1.13	b
w/nw	24	0.85	b
nw	63	0.29	c
nw/nw	30	0.17	c

Lycopersicon esculentum cv. T5 (*w*) is a chilling-sensitive tomato cultivar that wilts when its roots are chilled. Plants that are heterozygous for the *L. hirsutum* allele at *stm9* (*nw*) wilt only slightly, if at all, when their roots are chilled. Wilting scores are provided for ungrafted controls (*w* or *nw*), grafted controls composed of the shoots and roots from one phenotype (*w/w* or *nw/nw*), and grafted plants composed of a shoot from one phenotype noted before the slash and a root from another noted after the slash (*nw/w* or *w/nw*). A score of '3' indicates that the shoots were fully flaccid, whereas a score of '0' indicates that they were fully turgid. Phenotypes followed by different letters differed significantly according to a Bonferroni (Dunn) *t*-test ($P < 0.05$).

The genotypes differed in stomatal behaviour. Consequently, our current hypothesis on the differential chilling tolerance in these species follows that of Wilson (1976) for *Phaseolus vulgaris* : (a) low root temperatures immediately inhibit hydraulic conductance and, thereby, restrict water movement from the roots; (b) this rapid decline in water movement prompts the shoots of the chilling-tolerant species to close their stomata.

The wilting response of the grafted plants supports this scenario. As mentioned above, the grafting procedure itself did not have a significant effect on shoot turgor maintenance during root chilling because grafted plants with roots and shoots from the same phenotype (*nw/nw* or *w/w*) had the same response as the ungrafted plants of the same phenotype (*nw* or *w*) (Table 2). The two parents, *Lycopersicon esculentum* cv. T5 and *L. hirsutum* LA 1778, were graft-incompatible, but grafts between their progeny that maintained shoot turgor during root chilling and T5 were successful. Grafts between shoots and roots of different phenotypes (*nw/w* or *w/nw*) showed wilting responses intermediate between that of plants in which both the shoot and root were from T5 (*w/w*) and that of plants in which both the shoot and root were from the progeny (*nw/nw*) (Table 2). The wilting response of the grafted plants *nw/w* or *w/nw* were similar, indicating that both the shoot and root contribute to shoot turgor maintenance during root chilling.

Maize, another chilling-sensitive species, shows a response similar to tomato in that the variation in the chilling-tolerance among maize genotypes is correlated with their propensity to close stomata during chilling (Capell & Doerffling 1993; Perez, Irigoyen & Sanchez-Diaz 1997; Aroca *et al*. 2001, 2003). In maize, the E_a for hydraulic root conductance is about 9 (Aroca *et al*. 2001). Maize shoots suffer water stress in cold soils because the decline in tran-

spiration is not commensurate with the decreased water movement from roots (Capell & Doerffling 1993; Irigoyen, De Juan & Sanchez-Diaz 1996; Perez *et al*. 1997). Maize, unfortunately, is neither amenable to propagation by cuttings nor to grafting between shoots and roots; therefore, tomato has advantages over maize for sample replication and in separating the contributions of shoot and roots to the chilling response.

The nature of the root signal for stomatal closure during chilling is still unknown. Abscisic acid (ABA) is an obvious candidate (Wilkinson & Davies 2002; Dodd 2003). Root levels of ABA in tomato (Dale & Campbell 1981) and maize (Capell & Doerffling 1993) tend to increase under chilling as do shoot levels (maize, Janowiak, Luck & Dorffling 2003; tomato, Lee *et al*. 2003). Nonetheless, chilling tolerance and ABA levels are not strongly correlated in tomato (Bagnall *et al*. 1983; Li 1994) or maize (Ristic *et al*. 1998). Reciprocal grafts between the roots and shoots of wild-type tomato and ABA-deficient mutants demonstrated that stomatal behaviour is independent of a root's ability to produce ABA (Holbrook *et al*. 2002). ABA did not mediate cold-induced stomatal closure in *Commelina communis* (Wilkinson, Clephan & Davies 2001) or *Phaseolus vulgaris* (Vernieri *et al*. 2001). Cold-inducible freezing tolerance is unaffected in *Arabidopsis* mutants that are deficient in ABA synthesis or perception (Gilmour & Thomashow 1991). These results suggest that signals other than ABA are involved in the response to cold soils.

Several signals appear to be associated with ABA in regulating stomatal behaviour. Other phytohormones such as cytokinins interact with ABA (Dodd 2003). Alkalization of the xylem sap during drought may increase apoplastic ABA concentrations in leaves and promote stomatal closure (Wilkinson & Davies 1997; Wilkinson *et al*. 1998, 2001; Wilkinson 1999). ABA induces synthesis of nitric oxide in roots, and nitric oxide prompts stomatal closure (Guo, Okamoto & Crawford 2003). Polyamines such as putrescine may act independently of ABA (Kim *et al*. 2002).

Another possible explanation for stomatal behaviour during root chilling is that stomata are responding to changes in water status within the leaf as water flow declines (Cowan 1994; Saliendra, Sperry & Comstock 1995; Hubbard *et al*. 2001; Matzner & Comstock 2001). According to this hypothesis: (1) root chilling decreases the hydraulic conductance of the soil to leaf pathway causing a momentary decline in water status of at least a portion of the leaf tissue; (2) through pressure–volume changes in sensing cells or possibly transient cavitation within leaf veins, the change in water status provokes stomatal closure; (3) diminished stomatal conductance returns leaf water status to its original level; and (4) at the bulk tissue level, these small fluctuations in leaf water status in time and space are masked, and so bulk leaf water potential remains approximately constant.

Our data do not seem to fit easily into the framework of this hypothesis. When shoot water status was allowed to decline (Fig. 4a), stomatal conductance in both species decreased more than three-fold (Fig. 4c). Shoot water status, however, did not recover even several hours after stomatal closure (Fig. 4a). When the shoot water status of plants was held constant in the root pressure chamber, stomatal conductance in both species still declined with root temperature, but only by about 25%. Stomatal conductance of these plants did not recover even several hours after the return to moderate root temperatures (Fig. 5).

In summary, several parallel signal pathways between roots and stomata are likely to be operating during root chilling (Smallwood & Bowles 2002): one of which may involve ABA, another may involve leaf water status, and still others may involve additional factors.

The centre of origin for *L. esculentum* is the wet tropics of South America (Rick 1976). In such habitats, less restrained transpiration and lack of response to chilling-induced water deficits would not negatively influence reproductive fitness or productivity. Perhaps as a result, cultivated tomato transpires faster and with fewer environmental constraints than its wild relatives that are native to harsher environments such as deserts or high altitudes (Vallejos & Pearcy 1987; Torrecillas *et al*. 1995). Similarly, modern cultivars of Pima cotton and bread wheat have higher stomatal conductances that vary less with environmental changes than landraces of these crops (Lu *et al*. 1998). Cultivars selected for maximum yields under continuous and adequate inputs may lose the genetic ability to respond rapidly to environmental changes. This might provide the physiological basis for the differential chilling tolerance observed in *L. esculentum* and *L. hirsutum* .

REFERENCES

Ali I.A., Kafkafi U., Yamaguchi I., Sugimoto Y. & Inanaga S. (1996) Effects of low root temperature on sap flow rate, soluble carbohydrates, nitrate contents and on cytokinin and gibberellin levels in root xylem exudate of sand-grown tomato. *Journal of Plant Nutrition* **19,** 619–634.

Aroca R., Tognoni F., Irigoyen J.J., Sanchez-Diaz M. & Pardossi A. (2001) Different root low temperature response of two maize genotypes differing in chilling sensitivity. *Plant Physiology and Biochemistry* **39,** 1067–1073.

Aroca R., Vernieri P., Irigoyen J.J., Sanchez-Diaz M., Tognoni F. & Pardossi A. (2003) Involvement of abscisic acid in leaf and root of maize (*Zea mays* L.) in avoiding chilling-induced water stress. *Plant Science* **165,** 671–679.

Bagnall D., Wolfe J. & King R.W. (1983) Chill-induced wilting and hydraulic recovery in mung bean plants. *Plant, Cell and Environment* **6,** 457–464.

Bowers M.C. (1994) Environmental effects of cold on plants. In *Plant–Environment Interactions* (ed. R.E. Wilkinson), pp. 391–411. Marcel Dekker, New York, USA.

Capell B. & Doerffling K. (1993) Genotype-specific differences in chilling tolerance of maize in relation to chilling-induced changes in water status and abscisic acid accumulation. *Physiologia Plantarum* **88,** 638–646.

Cowan I.R. (1994) As to the mode of action of guard cells in dry air. In *Ecophysiology of Photosynthesis* (eds E.D. Schulze & M.M. Caldwell), pp. 205–229. Springer-Verlag, New York, USA.

Dale J.W. & Campbell R.F. (1981) Response of tomato plant to

stress temperature: increase in abscisic acid concentration. *Plant Physiology* **67,** 26–29.
Dalziel A.W. & Breidenbach R.W. (1982) Physical properties of mitochondrial lipids for *Lycopersicon hirsutum*. *Plant Physiology* **70,** 376–380.
Dodd I.C. (2003) Hormonal interactions and stomatal responses. *Journal of Plant Growth Regulation* **22,** 32–46.
Elmoazzen H.Y., Elliott J.A.W. & McGann L.E. (2002) The effect of temperature on membrane hydraulic conductivity. *Cryobiology* **45,** 68–79.
Epstein E. & Bloom A.J. (2004) *Mineral Nutrition of Plants: Principles and Perspectives*, 2nd edn. Sinauer Associates, Sunderland, MA, USA.
Fennell A. & Markhart A.H. (1998) Rapid acclimation of root hydraulic conductivity to low temperature. *Journal of Experimental Botany* **49,** 879–884.
Finkelstein A. (1987) *Water Movement Through Lipid Bilayers, Pores, and Plasma Membranes: Theory and Reality*, Vol. 4. Wiley, New York, USA.
Geisenberg C. & Stewart K. (1986) Field crop management. In *The Tomato Crop: a Scientific Basis for Improvement* (eds J.G. Atherton & J. Rudich), pp. 511–557. Chapman & Hall, London, UK.
Gilmour S.J. & Thomashow M.F. (1991) Cold-acclimation and cold-regulated gene-expression in ABA mutants of *Arabidopsis thaliana*. *Plant Molecular Biology* **17,** 1233–1240.
Guo F.Q., Okamoto M. & Crawford N.M. (2003) Identification of a plant nitric oxide synthase gene involved in hormonal signaling. *Science* **302,** 100–103.
Guy C.L. (1994) Low temperature and crop yield. In *Physiology and Determination of Crop Yield* (eds K.J. Boote, J.M. Bennett, T.R. Sinclair & G.M. Paulsen), pp. 417–424. SSSA, ASA, CSA, Madison, WI, USA.
Hales S. (1727) *Vegetable Staticks or, An account of some statical experiments on the sap in vegetables: being an essay towards a natural history of vegetation. Also, a specimen of an attempt to analyse the air, by a great variety of chymio-statical experiments; which were read at several meetings before the Royal Society.* W. & J. Innys, T. Woodward., London, England.
Holbrook N.M., Shashidhar V.R., James R.A. & Munns R. (2002) Stomatal control in tomato with ABA-deficient roots: response of grafted plants to soil drying. *Journal of Experimental Botany* **53,** 1503–1514.
Hubbard R.M., Ryan M.G., Stiller V. & Sperry J.S. (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant Cell and Environment* **24,** 113–121.
Irigoyen J.J., De Juan J.P. & Sanchez-Diaz M. (1996) Drought enhances chilling tolerance in a chilling-sensitive maize (*Zea mays*) variety. *New Phytologist* **134,** 53–59.
Janowiak F., Luck E. & Dorffling K. (2003) Chilling tolerance of maize seedlings in the field during cold periods in spring is related to chilling-induced increase in abscisic acid level. *Journal of Agronomy and Crop Science* **189,** 156–161.
Jung S., Steffen K.L. & Lee H.J. (1998) Comparative photoinhibition of a high and a low altitude ecotype of tomato (*Lycopersicon hirsutum*) to chilling stress under high and low light conditions. *Plant Science* **134,** 69–77.
Kaye C. & Guy C.L. (1995) Perspectives of plant cold tolerance: physiology and molecular responses. *Science Progress* **78,** 271–299.
Kim T.E., Kim S.-K., Han T.J., Lee J.S. & Chang S.C. (2002) ABA and polyamines act independently in primary leaves of cold-stressed tomato (*Lycopersicon esculentum*). *Physiologia Plantarum* **115,** 370–376.
Lee J.T., Prasad V., Yang P.T., Wu J.F., Ho T.H.D., Charng Y.Y. & Chan M.T. (2003) Expression of *Arabidopsis* CBF1 regulated by an ABA/stress inducible promoter in transgenic tomato confers stress tolerance without affecting yield. *Plant Cell and Environment* **26,** 1181–1190.
Li P.H. (1994) Crop plant cold hardiness. In *Physiology and Determination of Crop Yield* (eds K.J. Boote, J.M. Bennett, T.R. Sinclair & G.M. Paulsen), pp. 395–416. SSSA, ASA, CSA, Madison, WI, USA.
Lu Z.M., Percy R.G., Qualset C.O. & Zeiger E. (1998) Stomatal conductance predicts yields in irrigated Pima cotton and bread wheat grown at high temperatures. *Journal of Experimental Botany* **49,** 453–460.
Lyons J.M. & Raison J.K. (1970) Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury. *Plant Physiology* **45,** 386–389.
Marangoni A.G. & Stanley D.W. (1989) Phase transitions in microsomal membranes from chilling sensitive and chilling resistant tomato plants and fruit. *Phytochemistry* **28,** 2293–2301.
Markhart A.H., Fiscus E.L., Naylor A.W. & Kramer P.J. (1979) Effect of temperature on water and ion transport in soybean and broccoli systems. *Plant Physiology* **64,** 83–87.
Martin B. & Ort D.R. (1985) The recovery of photosynthesis in tomato subsequent to chilling exposure. *Photosynthesis Research* **6,** 121–132.
Matzner S. & Comstock J. (2001) The temperature dependence of shoot hydraulic resistance: implications for stomatal behaviour and hydraulic limitation. *Plant, Cell and Environment* **24,** 1299–1307.
Nishida I. & Murata N. (1996) Chilling sensitivity in plants and cyanobacteria: the crucial contribution of membrane lipids. *Annual Review of Plant Physiology and Plant Molecular Biology* **47,** 541–568.
Passioura J.B. & Munns R. (2000) Rapid environmental changes that affect leaf water status induce transient surges or pauses in leaf expansion rate. *Australian Journal of Plant Physiology* **27,** 941–948.
Patterson B.D., Paull R. & Smillie R. (1978) Chilling resistance in *Lycopersicon hirsutum* Humb. & Bonpl., a wild tomato with a wide altitudinal distribution. *Australian Journal of Plant Physiology* **5,** 609–617.
Pearce R.S. (1999) Molecular analysis of acclimation to cold. *Plant Growth Regulation* **29,** 47–76.
Perez D.J.J., Irigoyen J.J. & Sanchez-Diaz M. (1997) Chilling of drought-hardened and non-hardened plants of different chilling-sensitive maize lines changes in water relations and ABA contents. *Plant Science* **122,** 71–79.
Raison J.K. & Brown M.A. (1989) Sensitivity of altitudinal ecotypes of the wild tomato *Lycopersicon hirsutum* to chilling injury. *Plant Physiology* **91,** 1471–1475.
Rick C.M. (1976) Tomato *Lycopersicon esculentum* (Solanaceae). In *Evolution of Crop Plants* (ed. N.W. Simmonds), pp. 268–273. Longman, London, UK.
Ristic Z., Yang G., Sterzinger A. & Zhang L. (1998) Higher chilling tolerance in maize is not always related to the ability for greater and faster abscisic acid accumulation. *Journal of Plant Physiology* **153,** 154–162.
Saliendra N.Z., Sperry J.S. & Comstock J.P. (1995) Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. *Planta* **196,** 357–366.
Sassenrath G.F., Ort D.R. & Portis A.R. Jr (1990) Impaired reductive activation of stromal bisphosphatases in tomato leaves following low-temperature exposure at high light. *Archives of Biochemistry and Biophysics* **282,** 302–308.

Smallwood M. & Bowles D.J. (2002) Plants in a cold climate. *Philosophical Transactions of the Royal Society of London Series B-Biology Sciences* **357,** 831–846.

Steudle E. (1992) The biophysics of plant water: compartmentation, coupling with metabolic processes, and flow of water in plant roots. In *Water and Life: Comparative Analysis of Water Relationships at the Organismic, Cellular, and Molecular Levels* (eds G.N. Somero, C.B. Osmond & C.L. Bolis), pp. 173–204. Springer-Verlag, Heidelberg, Germany.

Stirzaker R.J. & Passioura J.B. (1996) The water relations of the root–soil interface. *Plant, Cell and Environment* **19,** 201–208.

Torrecillas A., Guillaume C., Alarcon J.J. & Ruiz-Sanchez C. (1995) Water relations of two tomato species under water stress and recovery. *Plant Science* **105,** 169–176.

Truco M.J., Randall L.B., Bloom A.J. & St. Clair D.A. (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*. *Theoretical and Applied Genetics* **101,** 1082–1092.

Vallejos C.E. & Pearcy R.W. (1987) Differential acclimation potential to low temperatures in two species of *Lycopersicon*: photosynthesis and growth. *Canadian Journal of Botany* **65,** 1303–1307.

Vallejos C.E. & Tanksley S.D. (1983) Segregation of isozyme markers and cold tolerance in an interspecific backcross of tomato (*Lycopersicon esculentum* × *Lycopersicon hirsutum*). *Theoretical and Applied Genetics* **66,** 241–247.

Venema J.H., Posthumus F., de Vries M. & van Hasselt P.R. (1999) Differential response of domestic and wild *Lycopersicon* species to chilling under low light: Growth, carbohydrate content, photosynthesis and the xanthophyll cycle. *Physiologia Plantarum* **105,** 81–88.

Venema J.H., Villerius L. & van Hasselt P.R. (2000) Effect of acclimation to suboptimal temperature on chilling-induced photodamage: comparison between a domestic and a high-altitude wild L*ycopersicon* species. *Plant Science* **152,** 153–163.

Vernieri P., Lenzi A., Figaro M., Tognoni F. & Pardossi A. (2001) How the roots contribute to the ability of *Phaseolus vulgaris* L. to cope with chilling-induced water stress. *Journal of Experimental Botany* **52,** 2199–2206.

Wilkinson S. (1999) pH as a stress signal. *Plant Growth Regulation* **29,** 87–99.

Wilkinson S. & Davies W.J. (1997) Xylem sap pH increase: a drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. *Plant Physiology* **113,** 559–573.

Wilkinson S. & Davies W.J. (2002) ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Plant, Cell and Environment* **25,** 195–210.

Wilkinson S., Clephan A.L. & Davies W.J. (2001) Rapid low temperature-induced stomatal closure occurs in cold-tolerant *Commelina communis* leaves but not in cold-sensitive tobacco leaves, via a mechanism that involves apoplastic calcium but not abscisic acid. *Plant Physiology* **126,** 1566–1578.

Wilkinson S., Corlett J.E., Oger L. & Davies W.J. (1998) Effects of xylem pH on transpiration from wild-type and flacca tomato leaves – A vital role for abscisic acid in preventing excessive water loss even from well-watered plants. *Plant Physiology* **117,** 703–709.

Wilson J.M. (1976) Mechanism of chill-hardening and drought-hardening of *Phaseolus vulgaris* leaves. *New Phytologist* **76,** 257–270.

Wolf S., Yakir D., Stevens M.A. & Rudich J. (1986) Cold temperature tolerance of wild tomato species. *Journal of the American Society for Horticultural Science* **111,** 960–964.

Yakir D., Rudich J. & Bravdo B.A. (1986) Adaptation to chilling: photosynthetic characteristics of the cultivated tomato and a high altitude wild species. *Plant, Cell and Environment* **9,** 477–484.

Zwieniecki M.A., Thompson M.V. & Holbrook N.M. (2002) Understanding the hydraulics of porous pipes: tradeoffs between water uptake and root length utilization. *Journal of Plant Growth Regulation* **21,** 315–323.

Received 4 March 2004; received in revised form 12 March 2004; accepted for publication 12 March 2004