Chilling injury (CI) caused by exposure of plants to low, but nonfreezing, temperatures has long been known to result in physiological dysfunction (Saltveit, 2000). While CI symptoms vary with plant species, cultivars, tissues, and growth stages, they generally develop rapidly after transfer of plants to nonchilling temperatures. Environmental factors such as light intensity, relative humidity and growth conditions prior to chilling can also affect the development of CI. Symptoms related to CI include reduced or retarded germination and seedling development, wilting, tissue chlorosis and necrosis, increased electrolyte leakage caused by altered membranes, imbalances in metabolism, and accelerated senescence (Kuk et al., 2003; Saltveit and Morris, 1990), all of which may impair optimum growth, development, and quality of susceptible horticultural crops.

Lyons (1973) proposed that membrane phase transitions observed at CI temperatures represented a primary physiological event that triggered the development of symptoms. For several good reasons, Saltveit (2000) in a comprehensive review of CI, effectively argued that a new paradigm would be more appropriate. The new paradigm replaces the phase transition as a single event with contributions of a number of factors each conferring a small level of tolerance to resistance. Saltveit (2000) argues that chilling tolerance evolved in diverse unrelated species, independently by a number of different mechanisms. Thus, it is more likely that several small changes accumulated in tolerant species, and that it would be more productive to sequentially identify and characterize mechanisms for these factors rather than search for a primary cause of chilling tolerance in all plants.

The occurrence of photo-oxidative stress and generation of reactive oxygen species (ROS) in plant tissues chilled for prolonged periods have been suggested as possible causes of impaired photosynthetic electron transport (Walker et al., 1991) and of membrane peroxidation in chilling-sensitive plants (Burden et al., 1994; Prasad et al., 1994; Wise and Naylor, 1987). ROS are also generated in plant cells under normal growth conditions, but damage is minimized by the production of antioxidants such as glutathione, beta-carotene, ascorbate, and/or numerous phenolic compounds. Antioxidative enzymes such as superoxide dismutase, ascorbate peroxidase, and glutathione reductase also serve to detoxify free radicals (Alscheter al., 1991). In support of the role of ROS in chilling tolerance, Kerdmaimongkol and Woodson (1999) demonstrated that transgenic tomato (Lycopersicon esculentum Mill.) ‘Ohio8245’ with reduced catalase activity, due to the ex-
pression of an antisense catalase gene, were more susceptible to CI than wild-type plants.

Soluble sugars and sugar alcohols are known to play important roles in protecting plants from stresses such as salinity, drought, and temperature (Ruthinasabapathi, 2000). A number of research reports have focused on the development of CI and its symptoms, but the role and composition of soluble sugars associated with chilling tolerance have been studied far less. A number of osmoprotectants such as glycine betaine, proline, reducing sugars, and sugar alcohols are known to be up regulated in response to various stresses. One suggested function is to scavenge free radicals (Hayashi et al., 1997; Kishor et al., 1995; Shen et al., 1997a).

Mannitol is a well-characterized sugar alcohol. It is a significant photosynthetic product in some plant species, and has been shown to increase following abiotic stress (Zamski et al., 2001). Mannitol has also been suggested to act as an osmoprotectant by reacting with damaging hydroxyl radicals to form mannitol radicals, which are then converted to mannose in the presence of oxygen (Franzini et al., 1994; Shen et al., 1997b). Transgenic tobacco (Nicotiana tabacum L.) plants that express the mannitol-1-phosphate dehydrogenase, mtlD, gene from E. coli accumulate increased levels of mannitol and are more tolerant of osmotic stress (Shen et al., 1997a, 1997b; Tarczynski et al., 1992). Studies with transgenic tobacco expressing a chloroplast localized mtlD suggest that mannitol protects from oxidative damage during drought and chilling stress by protecting thiol-regulated enzymes such as phosphoribulokinase, thioredoxin, ferredoxin, and glutathione from hydroxyl radical damage (Shen et al., 1997b).

While Bruggemann et al. (1999) found that over expression of glutathione reductase in transgenic tomato produced no difference in chilling sensitivity of the photosynthetic apparatus, Sen et al. (1993) reported that transferring a gene from pea for chloroplastic Cu/Zn superoxide dismutase improved tolerance to chill induced photoinhibition in tobacco. While more germane to freezing rather than CI, Pennycooke et al. (2003) demonstrated that down regulating α-galactosidase in ‘Mitchell’ petunias resulted in an increase in endogenous raffinose and improved freezing tolerance by 4 °C. Petunia and other CI susceptible horticultural crops are frequently grown in regions subject to cool spring and fall temperatures that can induce CI. Several horticultural species can benefit from gene modifications that impart potential to extend the growing season. Accordingly, we transformed a white-flowered nearly isogenic diploid petunia cultivar, ‘Mitchell’, and subjected the transformants to chilling stress to examine the impact of the mtlD gene on CI in petunia. The study reported here investigated chilling tolerance using transgenic petunia plants with low and high mannitol levels due to ectopic expression of the mannitol-1-phosphate dehydrogenase gene (mtlD). Its impact on whole-plant symptom expression, membrane leakage, osmotic potential, and chilling induced alteration of soluble carbohydrate metabolism is presented.

Materials and Methods

Transformation of Petunia Plants and Growth Conditions. Petunia xhybrida cv. Mitchell was transformed using a modified leaf-disk Agrobacterium tumefaciens (Smith & Towns.) Conn. (strain LBA 4404) cocultivation protocol published by Horsch et al. (1985). The genetic construct pCaMVMTLDS contained the 35S cauliflower mosaic virus promoter, the E. coli mtlD (mannitol-1-phosphate dehydrogenase) gene and a nopaline synthase termination signal subcloned into the binary vector pBin19 (Tarczynski et al., 1992). Plants of each T₅ transgenic line were allowed to flower and set seeds in a containment greenhouse. Two capsules of seed were collected from each transgenic line and germinated separately on MS medium (Schuler and Zielinski, 1989) containing 200 mg·L⁻¹ kanamycin. Numbers of green (kanamycin-resistance) and white (kanamycin-sensitive) seedlings (T₅) were counted after 3 weeks to test segregation for kanamycin resistance. Four transgenic lines (M2, M3, M8, and M9) segregated 3:1 (chi-square used as a statistical parameter) and were chosen for further use.

Expression of the nptII gene was confirmed in T₅ seedlings using an ELISA kit for the detection of neomycin phosphotransferase II (nptII, AHDIA, Elkhart, Ind.). Polymerase chain reaction (PCR) utilizing gene specific primers was conducted to confirm the presence of the mtlD transgene in the putative T₅ transformants following selection on kanamycin. Genomic DNA was extracted from leaves using a modified CTAB extraction method (Fulton et al., 1995). Fifty nanograms of genomic DNA was amplified using Taq Polymerase (Eppendorf, Hamburg, Germany) and 20 pmol each of the forward primer 5′GGGCGAGTGAACGTAAAAGAT3′ and the reverse primer 5′CATTTACGCGTGCTGAC3′. PCR was conducted for 35 cycles of denaturation at 92 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min with a Mastercycler gradient thermocycler (Eppendorf). The PCR products were visualized following electrophoresis on a 0.8% agarose gel stained with ethidium bromide (data not shown).

Vegetative cuttings from a selected T₅ plant of each transgenic line (M2, M3, M8, and M9) were rooted on MS media containing 100 mg·L⁻¹ kanamycin to provide plant materials for replicated chilling trials. All excised shoots (2 cm vegetative cuttings) of T₅ transgenic lines grew slower in the rooting medium containing 100 mg·L⁻¹ kanamycin than wild-type (WT) shoots grown in the kanamycin-free rooting medium. To compensate for this delay, excised shoots of T₅ transgenic lines were placed on the rooting medium containing kanamycin 4 d earlier than excised shoots of WT plants. Rooted plantlets of WT and T₅ transgenic lines were transferred to potting medium (Fafard #4; Conrad Fafard, Agawam, Mass.) after 2 weeks in tissue culture. Four- or 8-week-old plants containing one to two flower buds were used in all experiments.

Plant Growth and Analysis. WT and transgenic plants were grown in a containment growth chamber at 25 °C day/20 °C night, 12-h photoperiod, 60 μmol·m⁻²·s⁻¹. Plants in all experiments were watered regularly and fertilized weekly with 13.5 g·L⁻¹ Miracle Gro Nutriblend 21N–7.9P–14.9K (The Scotts Co., Marysville, Ohio). Each experiment was repeated three times. At 4 weeks, three plants of WT and transgenic M2, M3, M8, and M9 for each of the three replications were harvested and divided into shoots and roots. Dry weight and root : shoot ratios were determined after 7 d drying at 75 °C (Table 1).

Chilling Treatments. Eight-week-old plants of each line were separated into two groups. The control group (four plants for each line) remained in the growth chamber at 25 °C day/20 °C night with a 12-h photoperiod. The experimental chilled group was transferred to 3 ± 0.5 °C day/0 ± 0.5 °C night, 12-h photoperiod and 75% relative humidity for 3 weeks to induce chilling stress. Preliminary experiments were used to determine that 3 weeks chilling exposure provided better symptom expression than shorter intervals. After chilling treatments were completed, plants of both WT and transgenic lines were held at room temperature (25 ± 3 °C) for 24 h to develop symptoms. Visual injury, relative conductivity, osmotic potential, and carbohydrate content were then determined.
Table 1. Dry weight and root : shoot ratios of wild type Petunia x hybrida and transgenic petunias expressing the E. coli mtlD gene encoding mannitol-1-phosphate dehydrogenase (M2, M3, M8, M9). Plants were grown under nonchilling conditions at 25 °C day/20 °C night with a 12-h photoperiod. Data are means ± se of three plants. No significant differences were detected among means within columns based on Tukey’s Studentized mean comparison test, P<0.05.

| Plant line | Shoot (g dry wt) | Root (g dry wt) | Shoot ratio |
|------------|------------------|-----------------|-------------|
| WT         | 4.04 ± 0.20      | 0.99 ± 0.06     | 0.29 ± 0.02 |
| M2         | 3.57 ± 0.29      | 1.03 ± 0.09     | 0.31 ± 0.02 |
| M3         | 3.61 ± 0.18      | 1.00 ± 0.09     | 0.31 ± 0.01 |
| M8         | 3.83 ± 0.03      | 1.02 ± 0.11     | 0.28 ± 0.01 |
| M9         | 3.38 ± 0.24      | 0.99 ± 0.08     | 0.30 ± 0.02 |

Results

Four transgenic lines that segregated 3:1 for kanamycin resistance, as determined by the ability to germinate and grow on MS media containing 200 mg L⁻¹ kanamycin, were selected for further evaluation. The presence of the npd1 gene product was confirmed in these plants using ELISA (data not shown) and the mtlD gene was present in all kanamycin resistant T1 plants from the four lines selected as determined by PCR (data not shown). The mtlD gene was not detected in WT petunia plants (data not shown). While kanamycin delayed rooting in the transgenic plants compared to WT plants started on kanamycin-free media, growth rates were similar once plants were established in potting medium. Shoot and root dry weights of 4-week-old WT and transgenic plants did not differ significantly (Table 1).

Visual chilling symptoms of plants. Exposure of 8-week-old WT and T1 transgenic plants (M2, M3, M8, M9) to 3 weeks at 3 °C day/0 °C night (12-h photoperiod) resulted in visible leaf and whole-plant wilting after transfer to 25 ± 3.0 °C for 24 h. M3 and M8 regained turgor or remained turgid, and only a few leaves showed necrosis (Fig. 1). Eight-week-old plants were chosen for this experiment because this was the first stage at which flower-bud development was noticed. Flowering was suppressed (data not shown) and there were no visual differences in floral development among WT and transgenic lines during the chilling period.

Relative conductivity and membrane leakage of chilled plants. Expanded upper leaves of approximately the same size were collected 1 d after transfer of chilled plants to 25 ± 3.0 °C, and leaf samples of nonchilled plants were collected at the same time, directly from the 25 °C growth chamber. Leakage rates derived from time course regression analyses for relative conductivity of nonchilled control plants (WT, M2, M3, M8, M9) did not differ significantly (Fig. 2). However, relative conductivity of chilled transgenic lines M3 and M8 was significantly lower than WT, M2, and M9 (Fig. 3). Lower leakage rates were detected in chilled transgenic lines M3 and M8 compared to the low-mannitol containing transgenic and WT lines (Fig. 3), suggesting membrane stabilization occurred in the stressed high-mannitol containing lines.

Osmonic potential of chilled plants. Osmotic potential of leaf samples did not differ between WT and transgenic lines under nonchilling treatments, nor did they differ between WT and transgenic lines following chilling treatment (Fig. 4). However, the osmotic potentials of nonchilled plants were significantly higher (<0.4 MPa) when compared with those of chilled plants.

Soluble carbohydrates. No significant differences were detected in the inositol, glucose, fructose, sucrose, raffinose, or stachyose levels in control WT vs. transgenic plants. Mannitol was the only soluble sugar that differed in the nonstressed transgenic plants, and it was consistently higher in the mtlD transgenic lines M3 and M8 (Table 2). Mannitol content of transgenic lines M3 and M8 was ±3× that of WT and the transgenic lines designated M2 and M9. Mean mannitol levels of transgenic lines M2 and M9 were slightly higher than that of WT, but the differences were not significant. The other soluble carbohydrates measured did not differ between the low- and high-mannitol containing lines.

Carbohydrate metabolism was altered in plants exposed to chilling stress in both low- and high-mannitol containing lines. Except for raffinose, soluble carbohydrates of both WT and transgenic lines generally decreased following chilling. Even though mannitol levels of WT and all transgenic lines declined...
Fig. 1. Visual symptoms of chilling injury in wild type (WT) and transgenic petunia plants expressing the *E. coli* mtlD gene (lines M2, M3, M8, and M9) encoding mannitol-1-phosphate dehydrogenase. Photographs were taken after 24 h at 25 °C following a 21-d exposure to chilling stress at 3 °C/day and 0 °C night, with a 12-h photoperiod.

Fig. 2. Time course conductivity showing leakage rate of exosmosed cell sap from control, non-chill-stressed wild-type (WT) and *mtlD* transgenic (M2, M3, M8, and M9) petunia leaf disks. Leaf samples were collected and assayed after 22 d at 25 °C day/20 °C night with a 12-h photoperiod. Differences between slopes are not significantly different. Slopes and (r) are: M9 = 0.08 (0.6220); M2 = 0.0.07 (0.6913); WT = 0.08 (0.7291); M3 = 0.07 (0.5649); M8 = 0.07 (0.6650).

during chilling, the mannitol content of transgenic lines M3 and M8 remained ≈8x higher than WT, M2, and M9 following chilling treatment (Table 2). In addition, fructose, sucrose, and raffinose levels were consistently higher in the high-mannitol containing lines following chilling stress, while inositol, glucose and stachyose did not differ (Table 2).

**Discussion**

Tobacco plants have previously been transformed with the bacterial gene encoding mannitol-1-dehydrogenase (*mtlD*). While the expression of this gene in *E. coli* leads to mannitol catabolism, its ectopic expression in transgenic tobacco leads to the increased production and accumulation of mannitol (Tarczynski et al., 1992). These plants have provided a useful experimental system for studying the role of sugar alcohols in abiotic stress protection, and transgenic tobacco plants containing higher mannitol have increased tolerance to salt and drought stress (Shen et al., 1997a, 1997b; Tarczynski et al., 1992). Karakis et al. (1997) found that tobacco transformed with *mtlD* osmotically adjusted to salt stress, but not to drought stress. In their study the growth rate and size of transformed plants were reduced compared to control plants. In contrast, we did not detect any growth reduction in nonstressed petunia plants transformed with *mtlD* (Table 1).

While salinity and drought can be major limitations to crop productivity, chilling temperatures can also have a negative impact on horticultural crops. To investigate the role of mannitol during chilling stress, the *mtlD* gene from *E. coli* was transformed into petunia. Four transgenic lines containing the *mtlD* gene were identified. Two of those lines (high-mannitol lines; M3 and M8) had mannitol levels that were higher than nontransformed WT plants, while two transgenic lines (low-mannitol lines; M2 and M9) had mannitol levels similar to those detected in WT plants.

Following chilling stress treatments, the high-mannitol containing transgenic lines M3 and M8 had fewer chilling injury symptoms at 25 °C than the WT and low-mannitol containing transgenic lines M2 and M9. Only a few M3 or M8 plants wilted or had necrotic leaves when chilled compared to other lines. Death or stunted growth was observed in the chilled WT and in transgenic lines M2 and M9, but M3 and M8 showed continuous growth and some plants flowered ≈10 d after transfer to the greenhouse (data not shown). These visual observations were also consistent with leakage conductivity data, as the high-mannitol transgenic lines had less leakage following chilling. Electrolyte leakage from leaves is considered a reliable estimate of cell disruption and membrane integrity and is often used as a reliable measure of chilling injury (Kuk et al., 2003; Wolk and Herner, 1982).

Because ROS are known to cause membrane lipid peroxidation by increased formation of malondialdehyde and protein degradation by promotion of intra- and inter molecular cross-linking from protein fragmentation and disulfide bonding, it is not unreasonable to assume that a protective function of ROS scavengers may alleviate membrane disruption (Shen et al., 1997b; Stadtman, 1992). Although the accumulation site of mannitol in these transgenics was not investigated, better recovery and less leakage in the high mannitol containing lines suggests a possible role for mannitol in osmotic adjustment and should rather be considered as an osmoprotectant in these transgenic petunia lines.

According to Saltveit (2000), significant correlations between changes in chilling sensitivity and compositional changes in sugars and organic acids have not been found. The data reported here showed that chilling caused a shift of carbohydrate metabolism in WT and transgenic petunias (Table 2). However, a positive correlation between soluble sugar levels and chilling was not observed, unlike the significant correlations found between soluble carbohydrate levels in plants and their cold hardiness (Fischer and Holl, 1991; Imanishi et al., 1998; Stushnoff et al., 1993). Since chilling stress may or may not trigger cold acclimation, does not involve...
In this study inositol dropped and raf

Bohnert, H.J., D.E. Nelson, and R.G. Jensen. 1995. Adaptations to

Mannitol was the only soluble carbohydrate altered by trans-

Flowering was arrested in all lines during chilling (data not shown), similar to observations by Caulfield and Bounce (1988) and Wang and Baker (1979). In high-mannitol containing lines flowering resumed following the return to nonchilling temperatures. The incorporation of the mtlD gene into important bedding plants like petunia has the potential to extend their growing season, making it possible to plant earlier in the spring, as well as to maintain enhanced flower display later in autumn. While petunia was used as a model floriculture crop, this technology is potentially transferable to other crops and could be used to enhance chilling tolerance of a diverse array of annuals in the landscape provided gene expression and plant response outdoors is similar to that detected under controlled-environment conditions. The mtlD gene can be added to the list of metabolic regulators that may influence plant responses to chilling stress.

- Chilling

+ Chilling

ALSCHER, R.G., N.R. MADAMANCHI, AND C.L. CRAMER. 1991. Protective mechanisms in the chloroplast stroma. P. 145–155 In: E.J. Pell and K.L. Steffen (eds.). Active oxygen/oxidative stress and plant metabolism. Amer. Soc. Plant Physiologists, Rockville, Md.

ALLENDER, G.J., D.E. Nelson, AND R.G. JENSEN. 1995. Adaptations to environmental stresses. Plant Cell 7: 1099–1111.

BRUGGEMANN, W., V. BHEYEL, M. BRODKA, H. POTH, M. WEIL, AND J. STOCKHAUS. 1999. Antioxidants and antioxidative enzymes in wild-type and transgenic Lycopersicon genotypes of different chilling tolerance. Plant Sci. 140: 145–154.

Burden, R.H., V. Gill, P.A. Boyd, AND D. Okane. 1994. Chilling, oxidative stress and antioxidant enzyme responses in Arabidopsis thaliana. Proc. Royal Soc. Edinburgh 102B: 177–185.

Caulfield, F. AND J.A. BOUNCE. 1988. Comparative responses of photosynthesis to growth temperature in soybean (Glycine max L. Merril) cultivars. Can. J. Plant Sci. 68: 419–425.

Fischer, C. AND W. HOLL. 1991. Food reserves of scots pine (Pinus syl-

Franzini, E., H. Sellak, J. Hakim, AND C. Pasquier. 1994. Comparative sugar degradation by (OH) produced by the ion-driven Fenton reaction and gamma radiolysis. Arch. Biochem. Biophys. 309: 261–265.

Literature Cited

ALSCHER, R.G., N.R. MADAMANCHI, AND C.L. CRAMER. 1991. Protective mechanisms in the chloroplast stroma. P. 145–155 In: E.J. Pell and K.L. Steffen (eds.). Active oxygen/oxidative stress and plant metabolism. Amer. Soc. Plant Physiologists, Rockville, Md.

ALLENDER, G.J., D.E. Nelson, AND R.G. JENSEN. 1995. Adaptations to environmental stresses. Plant Cell 7: 1099–1111.

BRUGGEMANN, W., V. BHEYEL, M. BRODKA, H. POTH, M. WEIL, AND J. STOCKHAUS. 1999. Antioxidants and antioxidative enzymes in wild-type and transgenic Lycopersicon genotypes of different chilling tolerance. Plant Sci. 140: 145–154.

Burden, R.H., V. Gill, P.A. Boyd, AND D. Okane. 1994. Chilling, oxidative stress and antioxidant enzyme responses in Arabidopsis thaliana. Proc. Royal Soc. Edinburgh 102B: 177–185.

Caulfield, F. AND J.A. BOUNCE. 1988. Comparative responses of photosynthesis to growth temperature in soybean (Glycine max L. Merril) cultivars. Can. J. Plant Sci. 68: 419–425.

Fischer, C. AND W. HOLL. 1991. Food reserves of scots pine (Pinus syl-

Franzini, E., H. Sellak, J. Hakim, AND C. Pasquier. 1994. Comparative sugar degradation by (OH) produced by the ion-driven Fenton reaction and gamma radiolysis. Arch. Biochem. Biophys. 309: 261–265.
Table 2. Carbohydrate content (μmol·g^-1 dry weight) of wild-type (WT) Petunia ×hybrida and transgenic petunias expressing the E. coli mtlD gene encoding mannitol-1-phosphate dehydrogenase (M2, M3, M8, M9) subjected to nonchilling and chilling conditions. Control (nonchilled) plants were grown for 22 d under 25 °C day/20°C night with a 12-h photoperiod and chilled plants were grown for 21 d under 3 °C days and 0 °C nights with a 12-h photoperiod followed by 24 h at 25 °C.

| Chilling treatment | Group         | Inositol | Mannitol | Glucose | Fructose | Sucrose | Raffinose | Stachyose |
|--------------------|---------------|----------|----------|---------|----------|---------|-----------|-----------|
| No                 | WT            | 9.39 a   | 0.86 c   | 23.54 a | 42.95 a  | 23.75 a | 1.85 a    | 0.83 a    |
| No                 | Low-mannitol  | 9.78 a   | 1.14 c   | 23.13 a | 48.06 a  | 22.88 a | 2.53 a    | 0.65 a    |
| No                 | High-mannitol | 10.63 a  | 3.39 a   | 20.64 ab| 49.83 a  | 25.82 a | 2.5 a     | 0.64 a    |
| Yes                | WT            | 6.46 b   | 0.23 c   | 17.54 c | 36.43 b  | 11.25 b | 5.08 b    | 0.62 a    |
| Yes                | Low-mannitol  | 5.05 b   | 0.26 c   | 18.07 bc| 35.72 b  | 11.51 b | 5.06 b    | 0.45 a    |
| Yes                | High-mannitol | 4.58 b   | 2.18 b   | 20.08 ab| 50.22 a  | 19.33 a | 6.15 c    | 0.50 a    |

Data are means of three replications. Means followed by the same letter within each column are not significantly different at P < 0.05 using Tukey’s Studentized comparison test.

Fulton, T.M., J. Chunwongse, and S.D. Tanksley. 1995. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Mol. Biol. Rpt. 13:207–209.

Hamman, R.A. Jr., I.E. Dami, T.M. Walsh, and C. Stushnoff. 1996. Seasonal carbohydrate changes and cold hardiness of Chardonnay and Riesling grapevines. Amer. J. Enol. Viticult. 47:31–36.

Hayashi, H., L. Mustard, P. Deshmium, M. Ida, and N. Murata. 1997. Transformation of Arabidopsis thaliana with the codA gene for choline oxidase; accumulation of glycine betaine and enhanced tolerance to salt and cold stress. Plant J. 12(1):133–142.

Horsch, R.B., J.E. Fry, N.L. Hoffmann, D. Eichholtz, S.G. Rogers, and R.T. Fraley. 1985. A simple and general method for transferring genes into plants. Science 227:1229–1231.

Imanishi, H.T., T. Suzuki, K. Masuda, and T. Harada. 1998. Accumulation of raffinose and stachyose in shoot apices of Lonicera caerulea L. during cold acclimation. Scientia Hort. 72:255–263.

Karakis, B., P. Ozias-Akins, C. Stushnoff, M. Seufferheld, and M. Rieger. 1997. Salinity and drought tolerance of mannitol-accumulating transgenic tobacco. Plant Cell Environ. 20:609–616.

Kerdnaimongkol, K. and W.R. Woodson. 1999. Inhibition of catalase by antisense RNA increases susceptibility to oxidative stress and chilling injury in transgenic tomato plants. J. Amer. Soc. Hort. Sci. 124:330–336.

King, A.L., D.C. Joyce, and M.S. Roberson. 1988. Roles of carbohydrates in diurnal chilling sensitivity of tomato seedlings. Plant Physiol. 86:764–768.

Kishor, K.P.B., Z. Hong, G.H. Miao, C.A.A. Hu, and D.P.S. Verma. 1995. Overexpression of α-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol. 108:1387–1394.

Kuk, Y.I., J.H. Lee, H.Y. Kim, S.J. Chung, G.C. Chung, J.O. Guh, H.J. Lee, and N.R. Burgos. 2003. Relationships of cold acclimation and antioxidative enzymes with chilling tolerance in cucumber (Cucumis sativus L.). J. Amer. Soc. Hort. Sci. 128:661–666.

Lyons, J.M. 1973. Chilling injury in plants. Annu. Rev. Plant Physiol. 24:445–466.

Pennycooke, J.C., M.L. Jones, and C. Stushnoff. 2003. Down-regulating α-galactosidase enhances freezing tolerance in transgenic petunia. Plant Physiol. 133:901–909.

Prasad, T.K., M.D. Anderson, B.A. Martin, and C.R. Steward. 1994. Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. Plant Cell 6:65–74.

Purvis, A.C. and J.D. Rice. 1983. Low temperature induction of invertase activity in grapefruit flavedo tissue. Phytochemistry 22(4):831–834.

Rathinasabapathi, B. 2000. Metabolic engineering for stress tolerance: Installing osmoprotectant synthesis pathways. Ann. Bot. 86:709–716.