Plant Physiol. (1991) 97, 663–669
0032-0889/91/97/0663/07/$01.00/0

Received for publication January 15, 1991
Accepted June 17, 1991

Effects of Chilling on the Biochemical and Functional Properties of Thylakoid Membranes[1]

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ABSTRACT

The mechanism of chilling resistance was investigated in 4-week-old plants of the chilling-sensitive cultivated tomato, *Lycopersicon esculentum* Mill. cv H722, and rooted cuttings of its chilling-resistant wild relative, *L. hirsutum* Humb. and Bonpl., which were chilled for 3 days at 2°C with a 14-hour photoperiod and light intensity of 250 micromoles per square meter per second. This chilling stress reduced the chlorophyll fluorescence ratio, stomatal conductance, and dry matter accumulation more in the sensitive *L. esculentum* than in the resistant *L. hirsutum*. Photosynthetic CO_2 uptake at the end of the chilling treatment was reduced more in the resistant *L. hirsutum* than in *L. esculentum*, but recovered at a faster rate when the plants were returned to 25°C. The reduction of the spin trap, Tiron, by isolated thylakoids at 750 micromoles per square meter per second light intensity was taken as a relative indication of the tendency for the thylakoids to produce activated oxygen. Thylakoids isolated from the resistant *L. hirsutum* with or without chilling treatment were essentially similar, whereas those from chilled leaves of *L. esculentum* reduced more Tiron than the nonchilled controls. Whole chain photosynthetic electron transport was measured on thylakoids isolated from chilled and control leaves of the two species at a range of assay temperatures from 5 to 25°C. In both species, electron transport of the thylakoids from chilled leaves was lower than the controls when measured at 25°C, and electron transport declined as the assay temperature was reduced. However, the temperature sensitivity of thylakoids from chilled *L. esculentum* was altered such that at all temperatures below 20°C, the rate of electron transport exceeded the control values. In contrast, the thylakoids from chilled *L. hirsutum* maintained their temperature sensitivity, and the electron transport rates were proportionately reduced at all temperatures. This sublethal chilling stress caused no significant changes in thylakoid galactolipid, phospholipid, or protein levels in either species. Nonchilled thylakoid membranes from *L. hirsutum* had fourfold higher levels of the fatty acid 16:1, than those from *L. esculentum*. Chilling caused retailoring of the acyl chains in *L. hirsutum* but not in *L. esculentum*. The chilling resistance of *L. hirsutum* may be related to an ability to reduce the potential for free radical production by close regulation of electron transport within the chloroplast.

Chilling injury is a physiological disorder that occurs when plant species of tropical or subtropical origin are exposed to temperatures ranging between 0 and 12°C (13). In tomato, a chilling-sensitive species, low temperatures can reduce plant vigor and yield and can shorten the effective length of the growing season (3, 4). Chilling sensitivity is particularly limiting to crop production during the early stages of the growing season, when both day and night temperatures can be below 10 to 12°C.

The combination of light and low temperatures enhances chilling injury (8, 10) and can persist even after optimal growth temperatures are restored (12). A hypothesis has been presented suggesting that during chilling in the light, there is a block or transient disruption of the photosystems that makes them "leaky" and results in the transfer of energy to molecular oxygen, thus forming activated oxygen species (16, 23). This production of oxygen-based free radicals may contribute to the degradation of D1-protein, the related phenomena of photoinhibition (19), and, ultimately, to enhanced peroxidative activity within the thylakoid membranes (22, 23). Chilling resistance or tolerance can be defined as the ability of a chilling-sensitive plant to withstand the strain of the stress, such as excess free-radical production, for a given period of time (8). A high-altitudinal accession of *Lycopersicon hirsutum* has been shown to be sensitive to low temperatures, but responds more slowly to the stress than ecotypes from lower altitudes (15, 17), and presumably commercial cultivars of *L. esculentum*.

The present study was undertaken to examine these two species of tomato showing varying levels of tolerance to a sublethal combination of light and low temperature. The comparison was based on the potential for free radical production, thylakoid membrane damage, Chl fluorescence emissions, and photosynthetic and plant growth parameters.

MATERIALS AND METHODS

Plant Material

Four-week-old plants of chilling-sensitive tomato, *L. esculentum* Mill. cv H722 and rooted cuttings of *L. hirsutum* Humb. and Bonpl. (LA 1363, elevation 3100 m, Alta Fortaleza, Ancash, Peru), were chilled in a modified freezer cabinet for 72 h at 2°C with a relative humidity of 80%. The plants were chilled under a 16 h photoperiod and PPFD 250 μmol $m^{-2}s^{-1}$. Nonchilled control plants were maintained in a growth cabinet at 25°/20°C day/night cycle under similar light and humidity conditions.

Measurement of Photosynthesis

Rates of photosynthesis were determined using a closed gas exchange system (Li-Cor model LI-6200). The Li-Cor system

[1] Supported by Natural Sciences and Engineering Research Council Cooperative Research and Development and H. J. Heinz of Canada Ltd. Grant CRD-8352.

measured and computed five observations per plant while a portion of a leaf was inside the chamber and calculated the initial values by linear regression. PPFD was 250 μmol $m^{-2}s^{-1}$ at a temperature of 25°C. Plants previously chilled at 2°C were allowed to warm to 25°C before photosynthetic measurements were obtained. Values for stomatal conductance and CO_2 measurements in the form of Ci:Ca[2] ratio were also obtained.

Measurement of Chl Fluorescence

From the Chl fluorescence induction curves, Fo and Fp fluorescence values were measured with a plant productivity fluorometer (model SF30, Richard Brancker Research Ltd., Ontario, Canada). Plants were dark adapted for 15 min and Chl fluorescence data were collected under dim green light.

Isolation of Chloroplast Thylakoid Membranes

Young, almost fully expanded leaves were harvested and thylakoid membranes were obtained essentially as described by Kee *et al.* (8). The ice-cold thylakoid isolation medium contained 50 mM Mes-KOH (pH 6.5), 10 mM KCl, 5 mM $MgCl_2$, 200 mM sorbitol, 15 mM β-mercaptoethanol, and 0.5% (w/v) BSA. Four grams of leaves were homogenized in 40 mL of the extraction buffer for two 5-s bursts in a blender and the slurry was filtered through 16 layers of cheesecloth. A pellet was obtained following centrifugation for 5 min at 3000*g*. The pellet was resuspended using a soft paint brush in 25 mL of an ice-cold resuspension medium containing 5 mM Mes-KOH (pH 6.5), 10 mM KCl, 3 mM $MgCl_2$, 200 mM sorbitol, 3 mM β-mercaptoethanol, and 1.0% (w/v) BSA. This suspension was passed through one layer of Kimwipe tissue and centrifuged for 5 min at 3000*g*. The thylakoid membranes were finally suspended to a Chl content of 1 to 2 mM in resuspension buffer.

Activated Oxygen Measurement

Activated oxygen production was measured by ESR with a Varian E-12 spectrometer, using Tiron (1,2-dihydroxybenzene-3,5-disulfonic acid) (14). Thylakoid membranes were isolated as described above except that β-mercaptoethanol was absent from the isolation and resuspension buffers. Chl levels were adjusted to 12 to 25 mg/mL, and 100 μL aliquots of thylakoid membranes were mixed with 100 μL of 20 mM Tiron and drawn into 100 μL capillary tubes sealed at one end. The sealed capillary tubes were inserted into a quartz sample holder in the microwave cavity. The signal of the Tiron semiquinone radical was observed in the dark at 20°C and the height of the signal was followed by a field frequency lock plotted as a function of time. After 2 min, the samples was illuminated with light at PPFD of 750 μmol $m^{-2}s^{-1}$ and the increase in the Tiron signal was monitored for 3 min before the light was turned off. Spectra were recorded at a microwave power setting of 6.5 mW, microwave frequency of 9.75 GHz, time constant of 50 s, and modulation amplitude of 1.0 gauss.

Measurement of Photosynthetic Electron Transport

The linear electron transport rate, involving both photosystems, was determined from the rate of aerobic oxidation of photoreduced methyl viologen as described by Kee *et al.* (8). The rates of light-saturated electron transport were measured with a Clark-type polarographic O_2 electrode from 5° to 25°C at 5° intervals. The O_2 electrode was calibrated at each temperature setting. The reaction mixture contained 50 mM Hepes-KOH (pH 7.5), 100 mM sorbitol, 5 mM KCl, 2.5 mM $MgCl_2$, 100 μM methyl viologen, 10 mM NH_4Cl, 0.03 μM

[2] Abbreviations: Ci, intercellular CO_2 concentration; Ca, atmospheric CO_2 concentration; Ea, Arrhenius activation energy; Fo, nonvariable fluorescence; Fp, peak fluorescence; ESR, electron spin resonance.

Table II. *Changes in the Rates of Photosynthetic CO_2 Uptake after Chilling for 72 h at 2°C and after a Subsequent 24 h Recovery at 25°C and PPFD of 250 μmol m^{-2} s^{-1}.*

Each value represents the mean of six plants. Two groups of six plants each were used to obtain nonchilled control values. Control values (± SE) were 9.21 ± 0.22 for *L. esculentum* and 9.99 ± 0.56 μmol CO_2 m^{-2} s^{-1} for *L. hirsutum*.

Species	Photosynthetic CO_2 Uptake (μmol m^{-2} s^{-1})	
	Chill	Chill + recovery
	% of control	
L. esculentum	57	68
L. hirsutum	37	63

Table I. *Effects of Chilling on the Chl Fluorescence ratio Fp:Fo, CO_2 ratio Ci:Ca and Stomatal Conductance of Attached Leaves of L. esculentum and L. hirsutum*

Each value represents the mean of four to six plants. The asterisk represents a significant difference from the appropriate control value based on a Student's *t* test ($P \leq 0.05$).

Species	Treatment	Fp:Fo[a]	Ci:Ca[b]	Stomatal Conductance
				cm s^{-1}
L. esculentum	Control	1.47	0.84	0.73
	Chill	1.14*	0.85	0.42*
L. hirsutum	Control	1.52	0.81	0.76
	Chill	1.32*	0.89*	0.49*

[a] Ratio of peak to initial Chl fluorescence. [b] Ratio of intercellular CO_2 concentration:atmospheric CO_2 concentration.

Table III. *Weight of Chilled L. esculentum and L. hirsutum Plants after 3 d Chilling at 2°C and a Subsequent 8 d Growth at 25°C Compared with Nonstressed Control Plants*

Each value represents the mean of 12 plants. Values in parentheses represent chill as percentage of control. The asterisk represents a significant difference from the appropriate control based on a Student's *t* test (*, $P \leq 0.05$; **, $P \leq 0.01$).

Species	Treatment	Fresh Weight	Dry Weight	Water Content
		g		*g/g dry wt*
L. esculentum	Control	20.09	1.97	9.2
	Chill	16.06** (80)	1.28** (65)	11.55* (125)
L. hirsutum	Control	11.84	1.40	7.46
	Chill	9.73* (82)	1.07* (76)	8.09 (108)

valinomycin, 300 units of superoxide dismutase, and thylakoid membranes equivalent to 15 μM Chl.

Activation Energy of Electron Transport

To calculate Ea, rates of electron transport were measured from 5° to 25°C at 5° intervals. Values were calculated as:

$$\text{Ea} = 2.303 \frac{(\log K_2 - \log K_1)R}{\frac{1}{T_1} - \frac{1}{T_2}}$$

where K_1 and K_2 equal the rates of electron transport at temperatures T_1 and T_2, respectively. T_1 and T_2 are the assay temperatures in °K, and R is the gas constant.

Chemical Analysis of Thylakoid Membranes

Lipids were extracted from isolated thylakoid membranes with chloroform:methanol (2:1, v/v) and analyzed for galactolipids and phospholipids. The galactolipids were measured with a phenol-sulfuric acid reaction with galactose as a standard (6). The phospholipid content of the extracts were measured as P_i after perchlorate oxidation using a Fiske-Subbarow reagent (Sigma Chemical Co.) and quantified colorimetrically (5). The total fatty acid content in the lipid extract was estimated following methylation with MethElute (Chromatographic Specialties) and analyzed by gas chromatography with a DB-225 fused-silica column (J & W Scientific). The free fatty acid levels were determined following silylation with *N,O*-bis(trimethylsilyl)trifluoroacetamide:pyridine (50:50, v/v) and analyzed by gas chromatography with a DB-17 fused-silica column (J & W Scientific). Heptadecanoic acid was used as an internal standard to quantify fatty acid concentrations. The protein content of the thylakoid pellet was determined spectrophotometrically with a BCA protein assay reagent (Pierce) using BSA as a standard. The Chl and carotenoid levels of whole leaves were determined spectrophotometrically following extraction in 80% (v/v) acetone according to Lichtenthaler (11). The production of aldehydes in the thylakoids was followed using thiobarbituric acid (1).

RESULTS

Chilling both *L. esculentum* and *L. hirsutum* plants for 3 d at 2°C under a PPFD of 250 μmol $m^{-2}s^{-1}$ reduced the Chl fluorescence ratio, Fp:Fo, reduced stomatal conductance, and

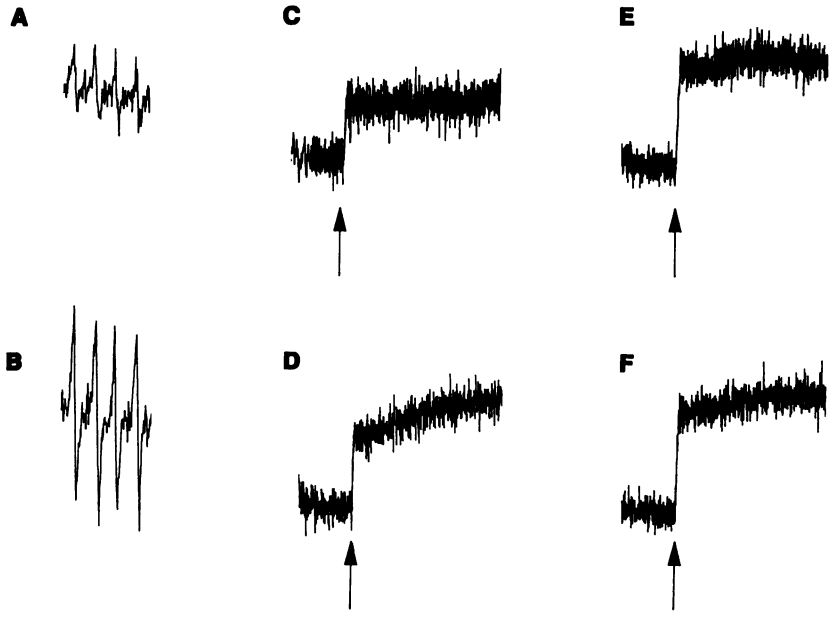

Figure 1. ESR spectra of the Tiron semiquinone radical. A, Free radical signal from nonchilled *L. esculentum* thylakoid membranes in the dark. B, Same preparation under 750 μmol $m^{-2}s^{-1}$ PPFD. Height of the Tiron signal was followed by a field frequency lock plotted as a function of time. The arrow represents the start of illumination at 750 μmol $m^{-2}s^{-1}$ PPFD. C, Nonchilled *L. esculentum* thylakoid membranes. D, Chilled *L. esculentum* thylakoid membranes. E, Nonchilled *L. hirsutum* thylakoid membranes. F, Chilled *L. hirsutum* thylakoid membranes. For comparison, all spectra were obtained with samples of equal Chl content.

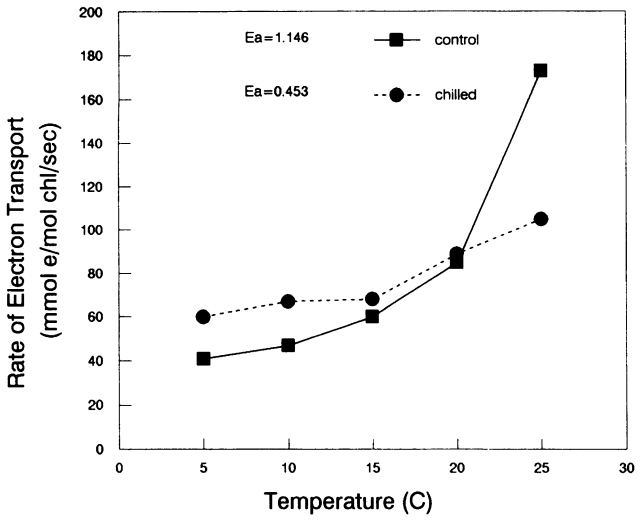

Figure 2. Effect of assay temperature on the rate of photosynthetic electron transport of thylakoid membranes isolated from leaves of chilled and nonchilled plants of *L. esculentum*.

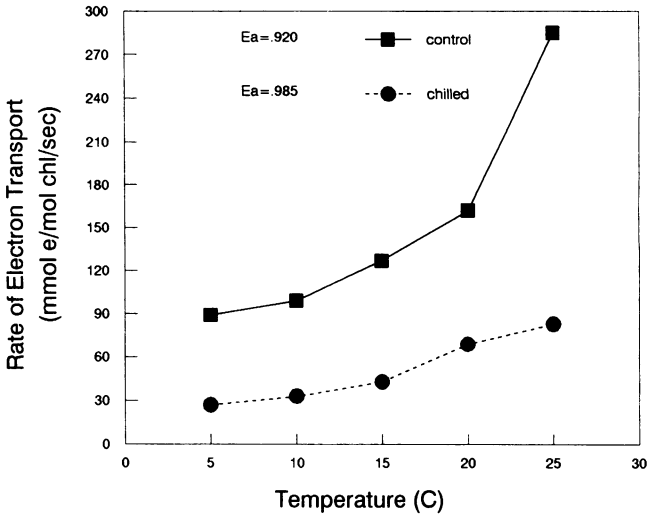

Figure 3. Effect of assay temperature on the rate of photosynthetic electron transport of thylakoid membranes isolated from leaves of chilled and nonchilled plants of *L. hirsutum*.

increased stomatal CO_2 content in *L. hirsutum* (Table I), which are all symptoms indicative of a stress response. Photosynthetic CO_2 uptake was reduced by 43% in the chilling-susceptible *L. esculentum* and, surprisingly, by 63% in the supposedly chilling-resistant *L. hirsutum* (Table II). Following 24 h recovery at 25°C, however, *L. hirsutum* had rapidly recovered photosynthetic activity to a level equivalent to *L. esculentum*. The drop in CO_2 fixation over this 3 d chilling period was less detrimental to the total dry matter accumulation of *L. hirsutum* than *L. esculentum* when measured 8 d after the low temperature treatment (Table III). Fresh weight was reduced similarly in the two species; however, there was a significant increase in the water content of *L. esculentum* shoots (Table III).

The relative amount of activated oxygen production by thylakoid membranes, as estimated by the amplitude of the Tiron signal from ESR, was minimal in the dark (Fig. 1A), but was substantial under 750 μmol $m^{-2}s^{-1}$ PPFD light (Fig. 1B). Thylakoid membranes from previously chilled *L. esculentum* leaves produced more activated oxygen than those from nonchilled plants and the rate of production increased with time in the light (compare Fig. 1C and 1D). In contrast, prior chilling caused only a slight increase in the rate of activated oxygen production by thylakoids from *L. hirsutum* (compare Fig. 1E and 1F). Because the Tiron signals were normalized to constant Chl, comparisons between the species are not possible with these spectra because the ratio of Chl to photosynthetic reaction centers may differ between the species. Similarly, the conclusion that chilling increases activated oxygen production is valid only if chilling does not reduce the Chl to reaction center ratio of *L. esculentum*.

Whole chain electron transport was measured on thylakoids isolated from control and chilled leaves of the two species as an alternative method of measuring the integrity of the thylakoid membrane system. The chilling treatment reduced whole chain electron transport (Figs. 2 and 3) when measured at 25°C in both *L. esculentum* and *L. hirsutum*. Although electron transport declined in all preparations as the assay temperature was reduced from 25 to 5°C, temperature sensitivity varied. The rate of electron transport in thylakoids from chilled leaves of *L. esculentum* was less responsive to changes in temperature than those from control leaves. Thus, Ea for control leaves was calculated as 1.15 and for chilled leaves as 0.45. As a result, below 20°C the rate of electron transport in thylakoids isolated from chilled *L. esculentum* was actually greater than in the control samples at the same temperature (Fig. 2). In contrast, in the chilling-tolerant *L. hirsutum*, Ea for electron transport was not significantly changed by the

Table IV. *Effects of Chilling on Galactolipid, Phospholipid, and Protein Levels in a Thylakoid Fraction Isolated from Leaves of L. esculentum and L. hirsutum*

Values not followed by the same letter within a column are significantly different at $P \leq 0.05$ ($n = 5$) according to a multiple range test.

Species	Treatment	Galactolipids	Phospholipids	Thylakoid Protein
		$\mu g\ g^{-1}$ dry wt		$mg\ g^{-1}$ dry wt
L. esculentum	Control	231 b	36.8 a	60 b
	Chill	219 b	41.2 a	71 b
L. hirsutum	Control	603 a	45.2 a	101 a
	Chill	463 a	43.6 a	102 a

Table V. *Effects of Chilling on Total Fatty Acid Composition (mol % of Total Fatty Acid) of Thylakoid Membranes Isolated from Leaves of Chilled and Nonchilled Plants of L. esculentum and L. hirsutum*

Values not followed by the same letter within a column are significantly different at $P \leq 0.05$ ($n = 4$) according to a multiple range test.

Species	Treatment	14:0	16:0	16:1	18:0	18:1	18:2	18:3
L. esculentum	Control	4 a	30 a	6 c	4 a	6 a	12 a	38 a
	Chill	4 a	29 a	6 c	5 a	6 a	14 a	36 a
L. hirsutum	Control	4 a	20 b	27 a	6 a	8 a	14 a	21 b
	Chill	4 a	20 b	17 b	4 a	6 a	11 a	38 a

chilling treatment (Ea = 0.92 and 0.99 for control and chilled, respectively) and, therefore, the rate of electron transport for the thylakoids from chilled *L. hirsutum* leaves was lower than the control samples at all assay temperatures.

Chilling treatments that induced changes in Chl fluorescence, stomatal conductance, photosynthesis, and growth did not significantly alter the recovery of galactolipid, phospholipid, or protein in the thylakoid membrane fraction from either species (Table IV). Comparing the two species, the thylakoid membrane fraction isolated from *L. hirsutum* had a significantly higher protein content than *L. esculentum*, but the amount of thylakoid phospholipid recovered was the same. Approximately 2.5 times more galactolipid was recovered from *L. hirsutum* than from *L. esculentum*. However, there was no difference in the quantity of total thylakoid fatty acids recovered on a dry weight basis between the two species (data not shown).

Thylakoid membranes from nonchilled *L. hirsutum* had approximately four times more 16:1 and concomitantly lower levels of 16:0 than membranes isolated from nonchilled *L. esculentum* (Table V). After 3 d of chilling, the recovery of total or free fatty acids did not change on a dry weight basis in either species (data not shown). However, there was some retailoring of the acyl chains in *L. hirsutum*, resulting in significant changes in the distribution of the fatty acids. The amount of 16:1 decreased and 18:3 increased, so that the overall distribution of fatty acids in *L. hirsutum* thylakoids more closely resembled that of *L. esculentum*. However, the level of 16:1 was still approximately three times higher in thylakoids from chilled *L. hirsutum* than in thylakoids from chilled *L. esculentum* (Table V). The distribution of total fatty acids, on a percentage basis, did not change significantly in thylakoids from *L. esculentum* plants as a result of the chilling treatment (Table V).

Plants exposed to 2°C for 3 d have slightly altered pigment profiles. The largest changes occurred in *L. hirsutum*, which showed a significant increase in carotenoids and total Chl levels, although the Chl *a*:Chl *b* ratio did not change (Table VI). The small changes observed in *L. esculentum* were not statistically significant ($P = 0.05$). If extensive lipid peroxidation had occurred in the thylakoid membranes during this chilling treatment, there should have been a decline in the polyunsaturated fatty acids, which was not observed (Table V). Similarly, an accumulation of thiobarbituric acid reactive species should have occurred in the chilled thylakoid membranes, but the values did not increase above control values (data not shown).

DISCUSSION

Increasing the relative level of chilling resistance in the cultivated tomato, *L. esculentum*, requires a clear understanding of the primary perturbations and long-term effects brought about by the combination of low temperature and light. Although many investigators have demonstrated that short-term exposure (*i.e.* less than 24 h) to low temperature and high light are detrimental to photosynthetic activity, exposure to chilling temperatures must be relatively long before cells of most sensitive plants are permanently injured (13). In tomato, the 2 to 3 week interval immediately following cotyledon expansion has been defined as the temperature-sensitive period determining when formation of the first inflorescence occurs (10, 24). A delay brought about by a relatively long-term exposure (*i.e.* 2–3 d) to day/night temperatures below 10°C would result in a reduction of the effective length of the growing season, fruit-set, and yield.

The time of chilling treatment in this study (*i.e.* 72 h) was of sufficient duration to cause a significant decline in both the fresh and dry weights of *L. esculentum* and, to a lesser extent, *L. hirsutum* (Table III). Chilling also caused a signifi-

Table VI. *Effects of Chilling on Chl and Carotenoid Levels ($\mu g\ g^{-1}$ Dry Weight) of Thylakoid Membranes Isolated from Leaves of Chilled and Nonchilled Plants of L. esculentum and L. hirsutum.*

Values not followed by the same letter within a column are significantly different at $P \leq 0.05$ ($n = 5$) according to a multiple range test.

Species	Treatment	Chl *a*	Chl *b*	$\frac{\text{Chl } a}{\text{Chl } b}$	Total Chl	Carotenoids
L. esculentum	Control	426 c	160 b	2.66	586 c	75 c
	Chill	466 bc	153 b	3.05	619 bc	68 c
L. hirsutum	Control	528 b	205 a	2.57	733 b	95 b
	Chill	658 a	253 a	2.60	912 a	130 a

cant increase in relative water content. According to the definitions of Raison and Lyons (18), *L. hirsutum* is classified as being more resistant to chilling because it is not affected to the same extent as *L. esculentum* with respect to measurements such as dry matter accumulation (Table III), Chl fluorescence (Table I, 21), recovery of photosynthetic activity after chilling (Table II), and rates of leaf elongation during low temperature growth (20, 25).

Wise and Naylor (22, 23) have suggested that the need for light and oxygen for the expression of chilling-induced injury may indicate that energy from the photosynthetic light reactions is diverted to molecular oxygen, resulting in the production of activated oxygen species, such as singlet oxygen or the superoxide radical. This hypothesis was investigated using several approaches. First, the spin trap, Tiron, was incubated with isolated thylakoids from chilled and control leaves of the two species. The observation that the ESR signal, and therefore production of activated oxygen, was greater in thylakoids from chilled *L. esculentum* than from the nonchilled control (Fig. 1) suggests that electron transport in the thylakoids from these chilled leaves was not as tightly regulated after chilling as it was in *L. hirsutum*.

A close regulation of electron transport at low temperatures to prevent uncontrolled production of activated oxygen within the chloroplast would appear, therefore, to be a mechanism of chilling resistance in *L. hirsutum*. This is supported further by the observation that whole chain electron transport rates are reduced proportionately at all temperatures in *L. hirsutum* exposed to chilling stress, whereas in *L. esculentum*, temperature sensitivity was altered dramatically after chilling, leading to increased electron transport at low temperatures (Figs. 2, 3). Similarly, photosynthetic CO_2 uptake (Table II) was reduced more in *L. hirsutum* compared with *L. esculentum* by the end of the chilling period, but recovery was more rapid. As previously suggested by Yakir *et al.* (25), this reduced photosynthetic capacity may account for some chilling resistance. In a whole plant situation, fluorescence quenching mechanisms would play an important role in electron transport regulation during a chilling stress, specifically by reducing the level of excess excitation energy within the chloroplasts. Bjorkman (2) has clearly shown that an increase in the rate constant for non-radiative energy dissipation, K_D, will reduce the amount of excess excitation energy and, therefore, is likely to have a protective role. However, there is a cost once the stress is relieved, in that failure to return to a state of low nonradiative energy dissipation would result in a decreased efficiency of photochemistry and, hence, a reduced efficiency of overall photosynthesis (2). Perhaps this is the situation with *L. esculentum*, which had significantly less dry matter accumulation following the chilling stress (Table III).

A second hypothetical mechanism of resistance to oxidative stress may involve the relative antioxidant capacity of *L. hirsutum* compared with *L. esculentum*. If a high titer of oxygen-centered free radicals or singlet oxygen is produced during chilling, the degradation of membrane lipids through peroxidation would be an anticipated consequence of chilling. This should be apparent as a decrease in the relative proportion of unsaturated fatty acids and losses of galactolipid, phospholipid, and pigments from thylakoid membranes. The lipid, pigment, and fatty acid analyses in this study (Tables IV–VI) indicate that lipid peroxidation could not be detected after this sublethal chilling treatment. Peroxidative damage (22, 23) and/or accumulations of free fatty acids (7) are certainly eventual consequences of chilling damage. However, the chilling stress used in the previous reports was administered to detached leaves or leaf disks under higher light intensities than those used in the current study.

The increased production of carotenoids in *L. hirsutum* after the chilling treatment (Table VI) is supportive of the hypothesis that oxidative stress is occurring during exposure to low temperatures. Carotenoids are known to deactivate triplet Chls and transform singlet oxygen to its triplet ground state (9), thereby reducing the potential for free radical production. A lack of evidence for the build-up of peroxidative by-products indirectly suggests that free radical scavengers are at sufficient levels to maintain membrane integrity. Higher light intensities and/or longer exposure times to low temperature may overwhelm the array of antioxidant systems within the chloroplast.

Finally, *L. hirsutum* has been used as a source of chilling tolerance in tomato by interspecific hybridization with *L. esculentum* (21). The results of this study would confirm previous observations (17) that *L. hirsutum* is chilling resistant, but not insensitive (according to the definitions in ref. 18), and that this resistance is achieved, at least in part, by a reduction of photosynthetic capacity, or in other words, growth, at low temperatures. As such, it would seem to be a relatively poor source for the genetic improvement of stress tolerance in the cultivated tomato in which the objective is to achieve not only survival but growth at low temperatures.

ACKNOWLEDGMENTS

Skilled technical assistance was provided by Ms. V. Oakley and Mr. B. Carrick. ESR facilities were kindly provided by Prof. E. G. Janzen, Department of Chemistry and Biochemistry. Assistance of Dr. D. McCullough with the Li-Cor 6200 is appreciated.

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