Photosynthetic thermotolerance is quantitatively and positively correlated with production of specific heat-shock proteins among nine genotypes of *Lycopersicon* (tomato)

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**Abstract**

We recently showed that the chloroplast small heat-shock protein (herein referred to as chlp Hsp24) protects photosystem 2 (PS2) during heat stress, and phenotypic variation in production of chlp Hsp24 is positively related to PS2 thermotolerance. However, the importance of chlp Hsp24 or other Hsps to other aspects of photosynthesis and overall photosynthetic thermotolerance is unknown. To begin investigating this and the importance of genetic variation in Hsp production to photosynthetic thermotolerance, the production of several prominent Hsps and photosynthetic thermotolerance were quantified in nine genotypes of *Lycopersicon*, and then the relationships between thermotolerance of net photosynthetic rate (*Pn*) and production of each Hsp were examined. The nine genotypes exhibited wide variation in *Pn* thermotolerance and production of each of the Hsps examined (chlp Hsp70, Hsp60, and Hsp24, and cytosol Hsp70). No statistically significant relationship was observed between production of chlp Hsp70 and *Pn* thermotolerance, and only a weak positive relationship between cytosolic Hsp70 and *Pn* was detected. However, significant positive relationships were observed between production of chlp Hsp24 and Hsp60 and *Pn* thermotolerance. Hence natural variation in production of chlp Hsp24 and Hsp60 is important in determining variation in photosynthetic thermotolerance. This is perhaps the first evidence that chlp Hsp60 is involved in photosynthetic thermotolerance, and these *in vivo* results are consistent with previous *in vitro* results showing that chlp Hsp24 protects PS2 during heat stress.

*Additional key words: chloroplast; heat stress; heat tolerance; photosynthesis; stress proteins.*

**Introduction**

We have recently shown that a chloroplast low-molecular-mass heat-shock protein (small Hsp) localizes to the thylakoid membranes, associates with the oxygen-evolving complex (OEC) proteins, and protects, but does not repair, photosystem 2 (PS2) during heat stress ( Heckathorn et al. 1998, 1999, Downs et al. 1999a,b). Further, when phenotypic variation in the chloroplast small Hsp is induced (e.g., by manipulating N availability), increased levels of Hsp are positively correlated with increased thermotolerance of PS2 (Stapel et al. 1993, Clarke and Critchley 1994, Heckathorn et al. 1996a). Also, greater production of the chloroplast small Hsp, both within (Park et al. 1996, Joshi et al. 1997) and among species (Downs et al. 1998), is positively correlated with whole-plant thermotolerance. In addition, a chloroplast Hsp of ca. 70 kDa (Hsp70) participates in protection or repair of PS2 during and after photoinhibition (Schroda et al. 1999), so chloroplast Hsp70 may play a similar role during heat stress. However, the importance of the chloroplast small Hsp and Hsp70 to other aspects of photosynthesis and overall photosynthesis is unknown, as is the importance of natural genetic variation in production of chloroplast small Hsp and Hsp70 to photosynthesis. Also, a role for other Hsps in photosynthetic thermotolerance has not yet been demonstrated.

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Abbreviations: C4 – internal CO2 concentration; chlp – chloroplast; Hsp – heat-shock protein; OEC – oxygen-evolving complex; *Pn* – net photosynthetic rate; PS2 – photosystem 2.

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To investigate the importance of the chloroplast small Hsp and Hsp70 to overall photosynthetic thermostolerance, and to begin exploring the potential importance of other Hsps to photosynthesis during heat stress, genetic variation in production of several prominent Hsps and photosynthetic thermostolerance was quantified in nine genotypes of *Lycopersicon*, and then the relationships between thermostolerance of net photosynthetic rate ($P_\text{N}$) and production of each Hsp were determined. In this study, four major Hsps were examined: cytosolic Hsp70(s) (cyto Hsp70), a 75-kD chloroplast Hsp (chlp Hsp70), the 60-kD chloroplast chaperonin (chlp Hsp60), and the chloroplast small Hsp. Several cytosol Hsp70 isoforms exist and constitutively expressed Hsp70s are thought to act as general chaperones, mediating correct folding of nascent proteins and shuttling of proteins to organelles (Parsell and Lindquist 1994). As with cytosol Hsp70, several chloroplast Hsp70 isoforms exist (Marshall et al. 1990, Wang et al. 1993, Bonk et al. 1996), and are thought to participate in the import of nuclear-encoded proteins into chloroplasts and subsequent folding of imported proteins (Yalovsky et al. 1992, Gatenby and Viitanen 1994, Bonk et al. 1997). Plastid Hsp60, in association with a 10-kD chaperonin, forms a large enzyme complex which helps mediate the correct assembly of ribulose-1,5-bisphosphate carboxylase/oxygenase and other chloroplast proteins (Goloubinoff et al. 1989, Lubben et al. 1989, Gatenby and Ellis 1990, Gatenby and Viitanen 1994, Bonk et al. 1997). In general, Hsps may limit damage to cellular components incurred by various stresses (not simply heat) or facilitate repair or degradation of other proteins after stress (Vierling 1991, Howarth and Ougham 1993, Parsell and Lindquist 1994, Waters et al. 1996, Downs and Heckathorn 1998, Heckathorn et al. 1998). As mentioned above, chlp small Hsp protects PS2, and consequently, whole-chain photosynthetic electron transport during heat stress (Heckathorn et al. 1998).

The synthesis and accumulation of different Hsps varies independently in response to stress in plants (i.e., each Hsp is independently regulated, cf. Howarth and Ougham 1993, Heckathorn et al. 1996a,b). Therefore, significant positive or negative relationships between photosynthetic thermostolerance and production of either chlp Hsp60, chlp Hsp70, or cyto Hsp70 would be some of the first evidence that Hsps other than the chloroplast small Hsp are involved in photosynthetic thermostolerance, and a significant positive relationship between the chloroplast small Hsp and thermostolerance of $P_\text{N}$ would provide *in vivo* evidence to support recent *in vitro* results. Further, we have an incomplete understanding of the importance of natural variation in Hsp production and the extent to which this contributes to variation in organismal thermostolerance and the distribution of species (Coleman et al. 1995). Relationships between natural genetic variation in Hsp production and thermostolerance would suggest that variation in production of Hsps is a trait on which natural selection can act, and thus would yield insight into the ecological and evolutionary importance of Hsps.

Materials and methods

**Plants and growth conditions:** We used nine genotypes of *Lycopersicon esculentum* acquired from the C.M. Rick Tomato Genetics Resource Center (University of California-Davis, CA, USA): *L. esculentum* Mill. cvs. Condine Red, Edkawi, Fireball, Gardener, Nagcarlang, Malintka, and Saladette; *L. esculentum* var. *cerasiforme* (Santa Cecilia), from Napo, Ecuador; and *L. chilense* var. Huaco Moquegua, from Moquegua, Peru. Tomato was used as a representative C$_3$ dicot because genotypes known to vary in thermostolerance are readily obtainable, and tomato Hsp production is well characterized in both commercial and wild genotypes (O’Connell 1994).

Twelve plants of each of the nine genotypes were grown from seed in commercial top soil, calcined clay, and sand (1:1:1; v:v:v) in 15-cm diameter × 15-cm height pots. Plants were raised in a greenhouse at Syracuse University under naturally fluctuating temperatures (15–30 °C) with ca. 12-h days and 12-h nights. Natural irradiance was supplemented with 200 μmol m$^{-2}$ s$^{-1}$ photosynthetic photon flux density (PPFD) provided by sodium-vapor lamps. Plants were watered daily and fertilized only once (at 1 week of age) with a dilute commercial NPK solution (Peter’s 20/20/20; Peter’s, Milpitas, CA, USA). Previous studies (Heckathorn et al. 1996a,b) indicated that nutrient-limited plants exhibited increased photosynthetic sensitivity to heat stress, thereby facilitating the divergence, quantification, and ranking of photosynthetic thermostolerance among genotypes. When the plants were 68 d-old, they were transferred to growth chambers (24 °C days and 18 °C nights; 400 μmol m$^{-2}$ s$^{-1}$ PPFD). Following a 36-h acclimation period, eight of the twelve plants of each genotype were heat stressed by gradually increasing the chamber temperature over two hours to 42 °C, holding this temperature for six hours, and then gradually decreasing temperature over two hours, back to control conditions; meanwhile, the four control plants were maintained throughout at 25 °C. During heat stress, the plants were kept well watered, and the growth chambers were humidified by misting (preliminary experiments indicated that this prevented water stress during heat stress treatments); leaf temperature was monitored with fine-wire thermocouples and a data logger.

**HSP content:** Leaf tissue (two leaflets from the second, most recently expanded leaf) was collected from each
plant for Hsp analysis immediately prior to, and at 12 and 24 h following, the onset of the heat stress treatment (n = 4 plants for each time point). Hsp content typically reached maximum levels at ca. these times in past experiments (Heckathorn et al. 1996a,b). Leaf samples from the four replicate plants at each harvest were pooled, and Hsp content was determined in triplicate for pre-heat-stress samples and in duplicate for the remaining samples as described previously (Heckathorn et al. 1996a,b, Downs et al. 1998). Briefly, leaf tissue was frozen in liquid N₂ immediately after harvest and stored at -20 °C. Total leaf protein was extracted by grinding leaves in liquid N₂ and then in buffer containing: 2% sodium dodecyl sulfate detergent (SDS), 150 mM TRIS-HCl (pH 7.8), 0.01% dithiothreitol (m/v), 4% polyvinylpyrrolidone (PVP; m/v), 2% polyvinyl-poly-pyrrolidone (PVPP; m/v), 1 mM phenylmethyl-sulfonyl fluoride, 3 mM EDTA, 1 mM benzamide, 1 mM s-aminocaproic acid, 2 mg per cm² leupeptin, 2 mg per cm² antipain, and 1% ascorbate (m/v). Protein concentration of each sample was determined in triplicate (as in Ghosh et al. 1988). Equal amounts of total protein (40 µg per lane) of each sample were fractionated by SDS polyacrylamide gel electrophoresis (SDS-PAGE; 16x16x 0.15 cm, 15% gels) and then transfused electrophoretically to polyvinylidene fluoride (PVDF) membranes. Proteins bound to the PVDF membrane were probed with protein-specific primary antibodies and then detected using secondary antibodies conjugated to alkaline phosphatase and nitroblue tetrazolium/5-bromo 4-chloro 3-indolylphosphate (NBT/BCIP). The relative amount of bound antibody was quantified by colorimetric densitometry, using a desktop scanner and NIH imaging software.

Within each genotype, the relative increase in Hsps in response to heat stress was quantified by normalizing Hsp content at each of the three harvests to pre-heat-stress levels (harvest 1); this was done separately for each Hsp (excluding Hsp24, since it was not constitutively expressed). To compare Hsp levels across genotypes, Hsp content of all nine genotypes was then normalized to the lowest pre-heat-stress value among the nine genotypes; again, this was done separately for each Hsp. We then compared maximum content (irrespective of when this occurred) among the genotypes for each Hsp, including Hsp24 (using separate gels containing replicate aliquots of one sample from each genotype). To ensure accurate estimates of relative protein content, triplicate lanes of pre-heat-stress samples were run on each gel, while duplicate lanes of other samples were run on each gel. Preliminary gels containing serial dilutions of leaf protein samples were run to ensure that protein content of each Hsp remained within the linear range of the protein content-densitometry relationship.

The relative content of the 70-kD cytosol Hsp(s) (cyto Hsp70), the chloroplast 75-kD Hsp (chlp Hsp70), chlp Hsp60, and the chloroplast small methionine-rich Hsp (ca. 24 kD in this study, hence chlp Hsp24) was quantified. The antibody to cyto Hsp70 which we used (SPA-820, StressGen, Victoria, BC, Canada) detects multiple members (constitutive and heat-inducible, and cytosolic, mitochondrial, chloroplast, etc.) of the Hsp70 group of proteins, of which the cytosolic forms are usually the most abundant (Vierling 1991, Howarth and Ougham 1993, O'Connell 1994). These multiple proteins have very similar masses and were not resolvable on one-dimensional gels in this study. Comparison of proteins detected by this antibody in whole-leaf vs. purified intact chloroplasts (as in Downs et al. 1998) indicated that this antibody primarily detected non-chloroplast Hsps in the whole-leaf samples that were examined (not shown). Therefore, the Hsps detected by this antibody are referred to simply as cyto Hsp70. The antibody to chlp Hsp70 which was used (MA3-007, Affinity Bioreagents; Golden, CO, USA) detected the same multiple, mostly non-chloroplast Hsp70s as above, but also detected a 75-kD chlp Hsp70 which was well resolved from the other Hsp70s (again confirmed with isolated chloroplasts). Anti-chlp Hsp60 antibody was obtained from StressGen (SPA-804) and detected negligible mitochondrial Hsp60, relative to chlp Hsp60, in whole-leaf samples. Anti-Hsp24 antibody, which is specific to the chloroplast small Hsp, was produced as in Downs et al. (1998), except that the antibody was generated using rabbits and an oligopeptide antigen with a slightly different amino-acid sequence (NH₂-Asp-Ile-Ser-Pro-Phe-Gly-Leu-Leu-Asp-Pro-Met-Ser-Pro-Met-lg-Thr-Met-Arg-Gly-Met-Leu-Asp-Met-Met-Asp-Arg-Met-Phe-Glu-Asp-Thr-COOH).

Photosynthesis measurements: P₅ was monitored before, during, and after heat stress as described in Heckathorn et al. (1996a). P₅ was measured as net CO₂ assimilation in the growth chamber, using a portable photosynthesis system (model 6200, LICOR, Lincoln, NE, USA), at an ambient CO₂ concentration of 390±10 cm³ m⁻², an irradiance of 400±10 µmol m⁻² s⁻¹ PPFD, and either 25 or 42 °C. P₅ values were collected from the most recently expanded leaves of intact plants (i.e., the leaves adjacent to those sampled for Hsps). Measurements were made repeatedly on the same plant throughout the experiment, and values were collected from both heat-stressed and control plants at each time point. P₅ thermotolerance was estimated by calculating the ratio of P₅ in heat-stressed plants to P₅ in control plants at each point in time. Leaf area was determined with a leaf-area meter (LiCOR).

Data analysis: Due to the high cost and limited supply of protein-specific antibodies, pooled samples were used for protein analysis, which precluded statistical analysis of protein data. However, to demonstrate the reproducibility of our Hsp determinations, coefficients of variation (CV) were calculated for the triplicate Hsp values of pooled
samples from the pre-heat-stress harvest. Among the nine genotypes, CVs averaged 8.1% for chl Hsp24, 12.6% for chl Hsp60, 12.0% for chl Hsp70, and 11.8% for cyto Hsp70; with the exception of one genotype, CVs did not exceed 20% for any Hsp. $P_N$ values were obtained from individual plants, so statistical analysis could be conducted on these data, which included linear regression analysis with associated correlation analysis and ANOVA.

**Results**

$P_N$ decreased in all genotypes during heat stress and remained below control levels in most genotypes during the two days after heat stress on which data were collected. To illustrate, results for a representative genotype, *L. esculentum* cv. Condine Red, are provided in Fig. 1. To determine the time-averaged effect of heat stress on $P_N$ during both the heat stress period and the following two days, which provided a more robust estimate of photosynthetic thermotolerance, we averaged the four heat-stress:control ratios (HS:C) obtained for each genotype (two during heat stress and one on each of the two following days) to obtain a single mean HS:C value (Table 1). Using time-averaged HS:C values as indicators, wide variation in the tolerance of $P_N$ to heat stress was observed among the nine tomato genotypes (Table 1), with HS:C values ranging from 0.68 to 0.96 (mean = 0.82). Decreases in $P_N$ in response to heat stress were not the result of increased stomatal limitation to photosynthesis. Although stomatal conductance ($g_s$) decreased somewhat in response to heat stress (not shown), estimated leaf internal CO$_2$ concentration (C$_i$) did not differ between heat-stressed plants and unstressed controls either during or after heat stress (Fig. 1), indicating that decreases in $g_s$ were proportional to decreases in $P_N$ and, thus, stomatal limitation to $P_N$ did not change with heat stress.

Table 1. The effects of acute heat stress on net photosynthetic rate, $P_N$ [μmol(CO$_2$) m$^{-2}$ s$^{-1}$] in nine genotypes of wild or cultivated tomato. Values are either means (+1 SE) for results from control and heat-stressed plants, or are the ratio of heat stress-to-control values (HS:C).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control</th>
<th>Heat stress</th>
<th>HS:C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. chilense</em></td>
<td>10.40 (0.45)</td>
<td>7.10 (0.74)</td>
<td>0.68</td>
</tr>
<tr>
<td><em>L. esculentum</em></td>
<td>10.40 (0.45)</td>
<td>7.70 (0.56)</td>
<td>0.74</td>
</tr>
<tr>
<td><em>L. esculentum</em></td>
<td>10.60 (0.46)</td>
<td>7.50 (0.52)</td>
<td>0.71</td>
</tr>
<tr>
<td><em>L. esculentum</em></td>
<td>9.90 (0.24)</td>
<td>9.40 (0.66)</td>
<td>0.95</td>
</tr>
<tr>
<td><em>L. esculentum</em></td>
<td>10.00 (0.57)</td>
<td>9.00 (0.35)</td>
<td>0.90</td>
</tr>
<tr>
<td><em>L. esculentum</em></td>
<td>9.20 (0.62)</td>
<td>8.80 (0.63)</td>
<td>0.96</td>
</tr>
<tr>
<td><em>L. esculentum</em></td>
<td>9.40 (0.37)</td>
<td>8.00 (0.54)</td>
<td>0.85</td>
</tr>
<tr>
<td><em>L. esculentum</em></td>
<td>10.60 (0.67)</td>
<td>9.50 (0.79)</td>
<td>0.90</td>
</tr>
<tr>
<td><em>L. esculentum</em></td>
<td>11.00 (0.51)</td>
<td>7.60 (0.38)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

As with photosynthetic performance, Hsp production varied widely among the nine tomato genotypes (Table 2). This was true for all four Hsps examined, whether Hsp production was quantified as the relative increase of a given Hsp within each genotype (ranging from 1.00 to 6.58) or the maximum content compared among genotypes (ranging from 1.00 to 9.81). Only maximum-content results (normalized to the lowest genotype) are presented for Hsp24. In contrast to chl Hsp70, chl Hsp60, and cyto Hsp70, which are all constitutively expressed but heat-responsive Hsps, chl Hsp24 was produced only in response to heat stress in these plants (as is common; Vierling 1991, Downs et al. 1998), so relative increases for this protein could not be calculated.

A significant positive relationship was observed between thermotolerance of $P_N$ and increased production of chl Hsp60 in response to heat stress, whether relative increase or maximum content of chl Hsp60 was regressed against $P_N$ thermotolerance (Fig. 2). No significant relationship was observed between chl Hsp70 production and $P_N$ thermotolerance, for either relative increase or maximum content. A marginally significant positive relationship was obtained for cyto Hsp70 vs. $P_N$ thermotolerance (relative-increase values...
only). For all three proteins in Fig. 2, regressions using relative-increase data yielded \( p \) and \( r^2 \) values that were nearly identical to those obtained when maximum-content data were used. As with chlp Hsp60, a significant positive relationship was observed between chlp Hsp24 production and \( P_N \) thermotolerance (Fig. 3).

Table 2. The effects of acute heat stress on production of chloroplast heat-shock protein 24 (chlp Hsp24), chlp Hsp60, chlp Hsp70, and cytosolic (cyto) Hsp70 in nine genotypes of wild or cultivated tomato. Shown are the relative increase of each Hsp in response to heat stress within each genotype and the relative maximum content of each Hsp compared across genotypes. Values are means of replicate measurements of pooled samples.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>chlp Hsp24 max rel. incr.</th>
<th>chlp Hsp70 max rel. incr.</th>
<th>cyto Hsp70 max rel. incr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. chilense</td>
<td>1.00 1.00</td>
<td>1.61 2.20</td>
<td>1.66 1.80</td>
</tr>
<tr>
<td>L. e. var. SC</td>
<td>1.63 3.07</td>
<td>4.74 1.31</td>
<td>3.10 3.50</td>
</tr>
<tr>
<td>L. e. cv. CR</td>
<td>1.31 1.61</td>
<td>2.49 1.70</td>
<td>3.10 2.88</td>
</tr>
<tr>
<td>L. e. cv. EDK</td>
<td>2.21 3.51</td>
<td>3.79 2.78</td>
<td>5.30 3.20</td>
</tr>
<tr>
<td>L. e. cv. FB</td>
<td>1.43 2.45</td>
<td>3.70 1.80</td>
<td>5.20 3.28</td>
</tr>
<tr>
<td>L. e. cv. GAR</td>
<td>2.71 6.58</td>
<td>9.81 2.72</td>
<td>4.80 5.35</td>
</tr>
<tr>
<td>L. e. cv. MAL</td>
<td>1.24 3.16</td>
<td>4.76 1.77</td>
<td>2.50 4.74</td>
</tr>
<tr>
<td>L. e. cv. NAG</td>
<td>2.19 3.03</td>
<td>6.97 2.39</td>
<td>7.80 3.72</td>
</tr>
<tr>
<td>L. e. cv. SAL</td>
<td>1.43 2.64</td>
<td>6.97 2.37</td>
<td>4.95 3.35</td>
</tr>
</tbody>
</table>

Fig. 2. Relationships between thermotolerance of net photosynthetic rate (\( P_N \)) and the relative increase in the content of specific heat-shock proteins (Hsps) in response to heat stress within each of nine genotypes of *Lycopersicon*, and relationships between \( P_N \) thermotolerance and maximum content of the same Hsps compared among genotypes. \( P_N \) thermotolerance was determined by calculating the time-averaged ratio of \( P_N \) in heat-stressed plants to \( P_N \) in control plants (see Materials and methods). The contents of chloroplast (chlp) Hsp60 (60 kDa), chlp Hsp70, and cytosolic (cyto) Hsp70 were normalized within each genotype to pre-heat-stress levels in determining relative increases and were normalized among and within genotypes (to the lowest pre-heat-stress level of the nine genotypes) for maximum content. Least-squares linear regression analysis was performed for each Hsp-thermotolerance relationship, and the resulting equation is shown, along with \( r^2 \) and \( p \) values from the associated correlation analysis and ANOVA.
Discussion

Among the nine *Lycopersicon* genotypes that were examined, wide variation was observed in thermotolerance of $P_N$ and production of chlp Hsp70, chlp Hsp60, chlp Hsp24, and cyto Hsp70 in response to heat stress. Importantly, natural variation in Hsp production was correlated with variation in photosynthetic thermotolerance, but only for certain Hsps. Significant positive relationships were observed between photosynthetic thermotolerance and production of chlp Hsp60 and chlp Hsp24, and a marginally significant positive relationship between cyto Hsp70 and photosynthetic thermotolerance was observed; however, no relationship was observed for chlp Hsp70. Within each Hsp, the strength of these relationships was very similar whether relative increase in Hsp content within each genotype or maximum Hsp content among genotypes was regressed against photosynthetic thermotolerance. Also, in the case of chlp Hsp24, the strength of these relationships were similar whether thermotolerance of $P_N$ or PS2 function alone was examined (see Heckathorn *et al.*, 1999 for PS2 vs. chlp Hsp24 results).

![Graph](image)

Fig. 3. The relationship between thermotolerance of net photosynthetic rate ($P_N$) and maximum content of the chloroplast small heat-shock protein (chlp Hsp24) for nine genotypes of *Lycopersicon*. $P_N$ thermotolerance and Hsp production were determined as described in Fig. 2. Least-squares linear regression analysis was performed for each Hsp-thermotolerance relationship, and the resulting equation is shown, along with $r^2$ and $p$ values from the associated correlation analysis and ANOVA.

These results are the first evidence that natural variation in certain Hsps, specifically chlp Hsp60, chlp Hsp24, and cyto Hsp70, is related to variation in photosynthetic thermotolerance. Since variation in Hsp production has an underlying heritable component (e.g., Frova and Gorla 1993, Park *et al.* 1996, Joshi *et al.* 1997, Krebs and Feder 1997), this study provides evidence that variation in photosynthetically important Hsps may be a trait on which natural selection can act or has acted. Furthermore, these results are some of the first evidence that specific Hsps, other than the chlp small Hsp, for which such evidence already exists, may play a role in determining photosynthetic thermotolerance.

As mentioned previously, direct in vitro evidence was recently obtained that the chlp small Hsp protects PS2 and the oxygen evolving complex at high temperatures (Heckathorn *et al.* 1998, Downs *et al.* 1999a), which confirmed the previous indirect evidence that this protein is involved in photosynthetic thermotolerance (Stapel *et al.* 1993, Clarke and Critchley 1994, Heckathorn *et al.* 1996a, Park *et al.* 1996, Joshi *et al.* 1997, Downs *et al.* 1998). Subsequently, a significant positive relationship between chlp Hsp24 and PS2 thermotolerance was observed ($r^2 = 0.78$, $p = 0.002$; Heckathorn *et al.* 1999). The previous in vitro studies cited above indicated that production of chlp Hsp24 completely accounted for acclimation of PS2, and thus whole-chain, electron transport in pre-heat-stressed tomatoes. The present study and in vivo results from *Agrostis palustris* (Heckathorn *et al.* 1999) provide in vivo confirmation that protection of PS2 by chlp Hsp24 is a major adaptation to heat stress in plants.

With the exception of chlp Hsp24, prior to this study there was little evidence that other Hsps are involved in photosynthetic thermotolerance. The results presented here suggest that chlp Hsp60 and cyto Hsp70 are also involved in determining photosynthetic thermotolerance. It has been argued that chlp Hsp60 is unlikely to play an important role in plant thermotolerance because content of this protein did not increase substantially with heat stress within a genotype in earlier studies (e.g., only a doubling with heat stress) (Vierling 1991, Viitanen *et al.* 1995). We observed relative increases in chlp Hsp60 content of greater than 600 % in the most thermostable genotype in this study, and several genotypes exhibited increases exceeding 300 %. Perhaps the present results reflect the tropical and subtropical origins of *Lycopersicon* or the fact that genotypes encompassing a wide range of thermotolerance were examined (previous studies focused on very thermosensitive species such as *Brassica napus*; Cloney *et al.* 1994, Viitanen *et al.* 1995).

Both chlp Hsp60 and cyto Hsp70 are constitutively expressed molecular chaperones (Gatenby and Viitanen 1994, Hartl 1996), but the specific function that these proteins fulfill in plants during or after heat stress is not known (Vierling 1991). In vitro, Hsp60 and Hsp70 from *E. coli* are able to protect proteins from denaturation during heat stress or reactivate damaged proteins following heat stress (e.g., Showyra *et al.* 1990, Höll-Neugebauer *et al.* 1991, Schröder *et al.* 1993), so that chlp Hsp60 and cyto Hsp70 may fulfill similar functions in plants. Also, cytosolic Hsp70s are involved in import of nuclear-encoded proteins into chloroplasts, so this may explain the significant correlation between photosynthetic thermotolerance and cyto Hsp70 content in the present study. The function of the 75-kD chlp Hsp examined in
this study is unknown (Wang et al. 1993), but the present results (i.e., no correlation) suggest that it may not play an important role in plant heat stress, but instead be important only during normal cell metabolism, such as shuttling of newly imported proteins to specific locations within the chloroplast (Bonk et al. 1996, 1997).

As in any organism, whole-plant thermostolerance is determined by the functional integration of many individual traits and adaptations. Unlike unicellular organisms which have only cellular-level metabolic adaptations available to them in responding to heat stress, plants and other complex multi-cellular organisms have tissue-, organ-, and organism-level adaptations upon which to draw as well. Perhaps higher-order adaptations that decrease heat gain or increase heat loss have rendered cellular adaptations less important or relegated cellular responses to the role of “last line of defense when higher-order adaptations have failed.” Such possibilities may explain in part why some studies have demonstrated a qualitative relationship between Hsp variation and variation in plant thermostolerance or habitat among genotypes or species (Ougham and Stoddart 1986, Krishnan et al. 1989, Park et al. 1996, Ristic et al. 1996, Joshi et al. 1997, Downs et al. 1998), while several other notable studies have observed no relationship between Hsps and thermostolerance among genotypes (Fender and O'Connell 1989, 1990, Frova and Gorla 1993, O'Connell 1994), suggesting that Hsps are not major determinants of organismal or photosynthetic thermostolerance in plants. Alternatively, relationships between Hsps and thermostolerance may be apparent only in some species, for certain Hsps, or for specific plant processes. Although the relative importance of cellular vs. higher-order adaptations to heat stress is not known, results from this study with tomatoes and similar results for fruit flies (Krebs and Feder 1997) suggest that, despite the evolution of higher-order adaptations, natural variation in Hsps is an important determinant of organismal variation in thermostolerance in plants and animals.

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