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

P.J. PRECZEWSKI, S.A. HECKATHORN*, C.A. DOWNS, and J.S. COLEMAN**

Department of Biology, Syracuse University, 130 College Place, Syracuse, NY, 13244, USA

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 (P_N) and production of each Hsp were examined. The nine genotypes exhibited wide variation in P_N 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 P_N thermotolerance, and only a weak positive relationship between cytosolic Hsp70 and P_N was detected. However, significant positive relationships were observed between production of chlp Hsp24 and Hsp60 and P_N 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.

Received 13 September 1999, *accepted* 28 February 2000.
*Fax: 315-443-2012, e-mail: saheckat@syr.edu
**Present address*: Desert Research Institute, 2215 Raggio Parkway, Reno, NV, 89512, USA.
Abbreviations: C_i – internal CO_2 concentration; chlp – chloroplast; Hsp – heat-shock protein; OEC - oxygen-evolving complex; P_N – net photosynthetic rate; PS2 – photosystem 2.
Acknowledgments: The authors thank M. Giovannini for help with data collection, and E.W. Hamilton, III, D. Barua, S.J. McNaughton, and the anonymous reviewers for comments. This work was supported by grants to JSC from the National Science Foundation (IBN-9207203 and IBN-9357302) and the Andrew W. Mellon Foundation. PJP was supported in part by a REU supplement from NSF. This publication arose from PJP's participation in the Syracuse University, Dept. of Biology's undergraduate research program.

To investigate the importance of the chloroplast small Hsp and Hsp70 to overall photosynthetic thermotolerance, 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 thermotolerance was quantified in nine genotypes of *Lycopersicon*, and then the relationships between thermotolerance of net photosynthetic rate (P_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 thermotolerance 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 thermotolerance, and a significant positive relationship between the chloroplast small Hsp and thermotolerance of P_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 thermotolerance and the distribution of species (Coleman *et al.* 1995). Relationships between natural genetic variation in Hsp production and thermotolerance 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. Huaico Moquegua, from Moquegua, Peru. Tomato was used as a representative C_3 dicot because genotypes known to vary in thermotolerance were 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 thermotolerance 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 N_2 immediately after harvest and stored at -20 °C. Total leaf protein was extracted by grinding leaves in liquid N_2 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 ε-aminocaproic acid, 2 mg per cm^3 leupeptin, 2 mg per cm^3 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; 16×16× 0.15 cm, 15 % gels) and then transferred electro-phoretically 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 resolveable 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 (NH_2-Asp-Ile-Ser-Pro-Phe-Gly-Leu-Leu-Asp-Pro-Met-Ser-Pro-Met-rg-Thr-Met-Arg-Gly-Met-Leu-Asp-Thr-Met-Asp-Arg-Met-Phe-Glu-Asp-Thr-COOH).

Photosynthesis measurements: P_N was monitored before, during, and after heat stress as described in Heckathorn *et al.* (1996a). P_N was measured as net CO_2 assimilation in the growth chamber, using a portable photosynthesis system (model *6200, LiCOR*, Lincoln, NE, USA), at an ambient CO_2 concentration of 390± 10 cm^3 m^3, an irradiance of 400±10 μmol m^{-2} s^{-1} PPFD, and either 25 or 42 °C. P_N 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. P_N thermotolerance was estimated by calculating the ratio of P_N in heat-stressed plants to P_N 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 chlp Hsp24, 12.6 % for chlp Hsp60, 12.0 % for chlp 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.

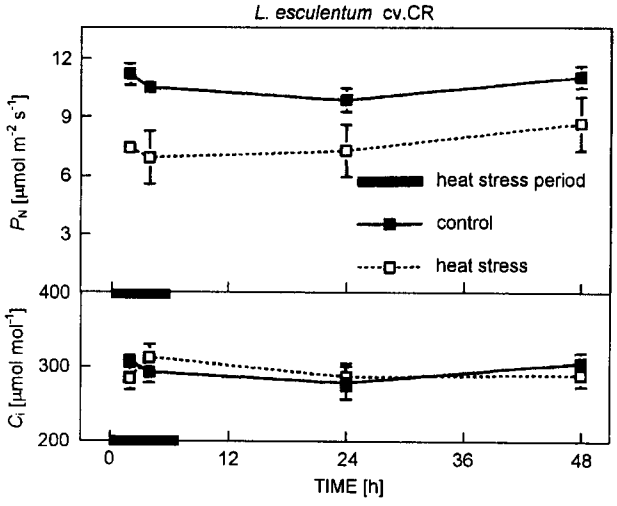

Fig. 1. Net photosynthetic rate (P_N = net CO_2 assimilation) and internal CO_2 concentration of leaves (C_i) in *Lycopersicon esculentum* Mill. cv. Condine Red during and after heat stress (6 h at 42 °C for heat-stressed plants only = *solid bar*). Results are from recently expanded leaves of both heat-stressed (*open symbols*) and unstressed control plants (*closed symbols*). P_N was measured in the growth chamber at 400 µmol m^{-2} s^{-1} PPFD at either 28 or 42 °C. Results are means ± 1 S.E.

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).

	Control	Heat stress	HS:C
L. chilense	10.40 (0.45)	7.10 (0.74)	0.68
L. esculentum var. SC	10.40 (0.45)	7.70 (0.56)	0.74
L. esculentum cv. CR	10.60 (0.46)	7.50 (0.52)	0.71
L. esculentum cv. EDK	9.90 (0.24)	9.40 (0.66)	0.95
L. esculentum cv. FB	10.00 (0.57)	9.00 (0.55)	0.90
L. esculentum cv. GAR	9.20 (0.62)	8.80 (0.63)	0.96
L. esculentum cv. MAL	9.40 (0.37)	8.00 (0.54)	0.85
L. esculentum cv. NAG	10.60 (0.67)	9.50 (0.79)	0.90
L. esculentum cv. SAL	11.00 (0.51)	7.60 (0.38)	0.69

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 chlp Hsp70, chlp Hsp60, and cyto Hsp70, which are all constitutively expressed but heat-responsive Hsps, chlp 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 chlp Hsp60 in response to heat stress, whether relative increase or maximum content of chlp Hsp60 was regressed against P_N thermotolerance (Fig. 2). No significant relationship was observed between chlp 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.

	chlp Hsp24 max	chlp Hsp60 rel. incr.	max	chlp Hsp70 rel. incr.	max	cyto Hsp70 rel. incr.	max
L. chilense	1.00	1.00	1.61	2.20	6.30	1.66	1.80
L. e. var. SC	1.63	3.07	4.74	1.31	3.10	3.50	3.72
L. e. cv. CR	1.31	1.61	2.49	1.70	3.10	2.88	3.22
L. e. cv. EDK	2.21	3.51	3.79	2.78	5.30	3.20	3.20
L. e. cv. FB	1.43	2.45	3.70	1.80	5.20	3.28	3.70
L. e. cv. GAR	2.71	6.58	9.81	2.72	4.80	5.35	8.97
L. e. cv. MAL	1.24	3.16	4.76	1.77	2.50	4.74	5.27
L. e. cv. NAG	2.19	3.03	6.97	2.39	7.80	3.72	5.56
L. e. cv. SAL	1.43	2.64	2.64	2.37	4.95	3.35	3.84

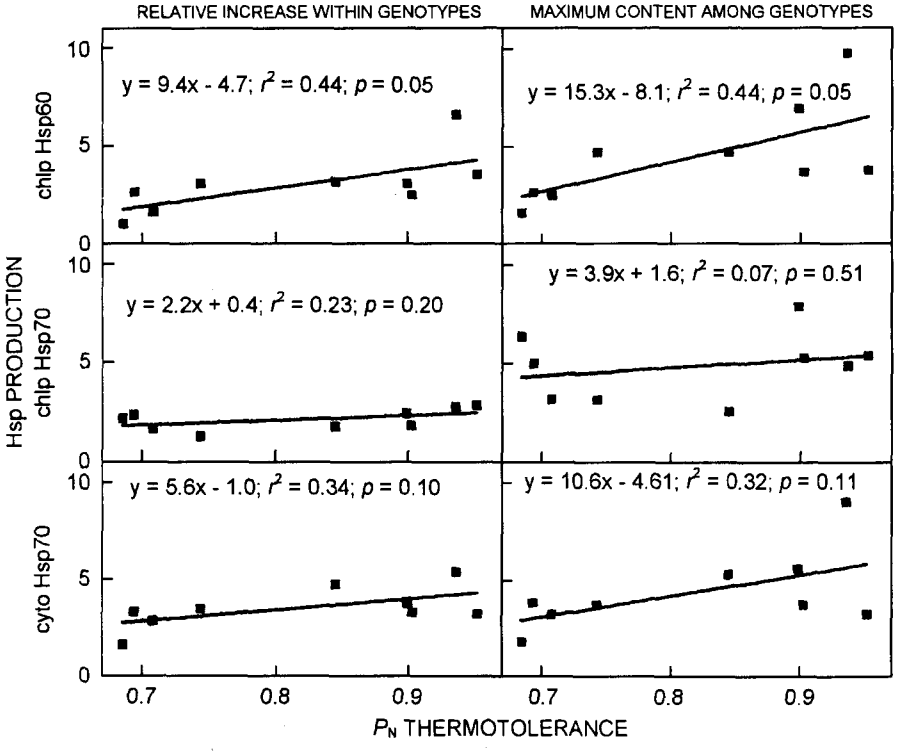

Fig. 2. Relationships between thermotolerance of net photosynthetic rate (P_N) and the relative increase in the content of specific heat-shock proteins (Hsps) in response to heat stress within each of nine genotypes of *Lycopersicon*, and relationships between P_N thermotolerance and maximum content 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).

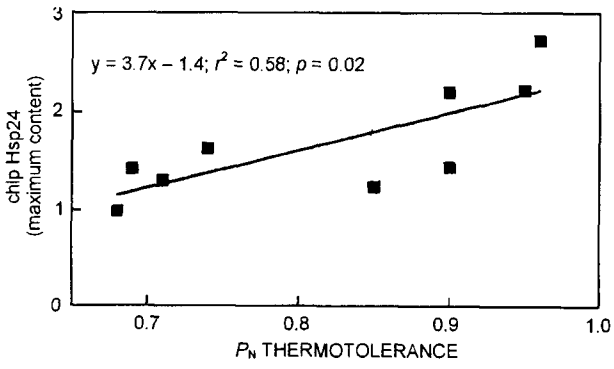

Fig. 3. The relationship between thermotolerance of net photosynthetic rate (P_N) and maximum content of the chloroplast small heat-shock protein (chlp Hsp24) for nine genotypes of *Lycopersicon*. P_N thermotolerance and Hsp production were determined as described in Fig. 2. Least-squares linear regression 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 thermotolerant 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 thermotolerance 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 thermotolerance 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 thermotolerance 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 thermotolerance in plants. Alternatively, relationships between Hsps and thermotolerance 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 thermotolerance in plants and animals.

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