Plant Physiol. (1990) 93, 1140–1146
0032-0889/90/93/1140/07/$01.00/0

Received for publication October 24, 1989
Accepted March 26, 1990

Expression of the Heat Shock Response in a Tomato Interspecific Hybrid Is Not Intermediate between the Two Parental Responses[1]

Susan E. Fender and Mary A. O'Connell*

Department of Agronomy and Horticulture and Plant Genetic Engineering Laboratory, New Mexico State University, Las Cruces, New Mexico 88003

ABSTRACT

While it is apparent that the heat shock response is ubiquitous, variabilities in the nature of the heat shock response between closely related species have not been well characterized. The heat shock response of three genotypes of tomato, *Lycopersicon esculentum*, *Lycopersicon pennellii*, and the interspecific sexual hybrid was characterized. The two parental genotypes differed in the nature of the heat shock proteins synthesized; the species-specific heat shock proteins were identified following *in vivo* labeling of leaf tissue with [^{35}S]methionine and cysteine. The duration of, and recovery from, heat shock varied between the two species: *L. esculentum* tissue recovered more rapidly and protein synthesis persisted longer during a heat shock than in the wild species, *L. pennellii*. Both species induced heat shock protein synthesis at 35°C and synthesis was maximal at 37°C. The response of the F1 to heat shock was intermediate to the parental responses for duration of, and recovery from, heat shock. In other aspects, the response of the F1 to heat shock was not intermediate to the parental responses: the F1 induced only half of the *L. esculentum* specific heat shock proteins, and all of the *L. pennellii* specific heat shock proteins. A discussion of the inheritance of the regulation of the heat shock response is presented.

The response of many organisms to elevated temperature has been characterized and described as the heat shock response (13). While it is apparent that the heat shock response is ubiquitous, variations in the nature of the heat shock response between closely related species have not been well characterized. The heat shock response in plants has been well characterized primarily in two crop plants, soybean (2, 8, 12) and corn (3, 4), and to a lesser extent in a few other systems (5, 7, 10, 16, 17, 23). There have been reports on the heat shock response of a cell culture of a wild species of tomato, *Lycopersicon peruvianum* (19, 20, 27) and a recent report on the tissue specific expression of an HSP70 cognate in cultivated tomato (6). However, very little information is available on the heat shock response of the cultivated tomato.

Tomato species represent excellent sources of genetic diversity, including tolerances to abiotic stresses (25). We have been investigating the cell genetics of tomato species, in particular of two species, *Lycopersicon esculentum*, the cultivated tomato, and *Lycopersicon pennellii*, a green-fruited, drought-tolerant, and water-use efficient species (18, 25). Sexual and somatic hybridization can produce interspecific hybrids between these two species (22, 24). We investigated the heat shock response in these two sexually compatible species to determine the range of variation of expression in the heat shock response and the nature of the expression of the response in the hybrid genotype. The parameters of the heat shock response that were measured included: the mol wt and isoelectric point of the HSPs[2] synthesized, the temperatures of induction and of maximal synthesis, the differences in duration of and recovery from the heat shock. All of these parameters were characterized in the interspecific F1 and compared with the parental responses.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Seeds of *Lycopersicon esculentum*, Mill. cultivar UC82L, were provided by Petoseed Co. Seeds of *L. pennellii* Cor. LA 716 were provided by Dr. Charles Rick, Tomato Genetics Cooperative, University of California, Davis. The sexual interspecific hybrid, F1, was produced in our greenhouse by hand emasculation of UC82L flowers and subsequent pollination with pollen from *L. pennellii*. The F1 plants were demonstrated to be hybrid based on a variety of morphological markers; since the parental plants were homozygous, all the F1 individuals were genetically identical. All plants used for analysis of the heat shock response were grown from seed in environmental growth chambers. The chambers were set for 16 h d, 25°C. The plants were watered with a nutrient solution based on Nitsch salts (21).

In Vivo Labeling, SDS-PAGE Analysis, Two-Dimensional Gels

The methods for labeling, SDS-PAGE, and two-dimensional gel analysis have been described (7). Young, fully expanded leaf tissue was used for *in vivo* labeling. Essentially, leaflets were held at the indicated temperatures in incubation

[1] This work was supported in part by the USDA Southwest Consortium on Plant Genetics and Water Resources, New Mexico State Agricultural Experiment Station, and the Rio Grande Research Corridor.

[2] Abbreviation: HSPs, heat shock proteins.

buffer (1% [w/v] sucrose, 1 mM potassium phosphate [pH 6.0], 50 μg/mL chloramphenicol) for 20 min, at which time the incubation buffer was spiked with [^{35}S]methionine and cysteine, 0.5 μCi/μL, (Trans Label, ICN, 1100 Ci/mmol), and the incubation was continued for the indicated period of time, typically 2 h. Samples were rinsed in cold water and ground in a mortar and pestle on ice with extraction buffer (2% [w/v] sodium dodecyl sulfate, 5% [v/v] β-mercaptoethanol, 62 mM Tris-HCl [pH 6.8], 10% [v/v] glycerol, 0.00125% [w/v] bromophenol blue). Samples were boiled for 2 min and insoluble debris removed by centrifugation in a microfuge. The incorporation of radioactive methionine and cysteine into proteins was measured following TCA precipitation as described by Mans and Novelli (15). Samples to be used for isoelectric focusing were precipitated with 9 volumes of ice-cold ethanol, and resuspended in isoelectric focusing loading buffer (1). For SDS-PAGE analysis, 10 to 15% (w/v) gradient polyacrylamide gels using a discontinuous buffer system (11), were used to analyze the *in vivo* labeled proteins. The second dimension of two-dimensional gels used the same SDS-PAGE system except that the separating gel was 12% (w/v) polyacrylamide. Gel lanes were loaded with equivalent amounts of TCA precipitable cpm. Low mol wt protein standards (BioRad) were used to calibrate the gels. Following electrophoresis, the gels were stained with Coomassie brilliant blue, destained, prepared for fluorography using Enhance (NEN/DuPont), dried between two sheets of cellophane film, and exposed to x-ray film. All of the results presented were observed in at least two independent replications.

Duration and Recovery Experiments

The synthesis of HSPs during a persistent heat shock was characterized by incubating leaf tissue at 35 or 37°C for 4, 8, 12, or 24 h. [^{35}S]Methionine and cysteine were added to the samples for a 1 h period immediately prior to collection of the sample for SDS-PAGE analysis as described above.

The recovery from HSP synthesis following a shift from heat shock temperatures to control temperatures was characterized by incubating leaf tissue at either 40 or 42°C for 2 h and then transferring the tissue to 25°C. The samples were incubated at 25°C for increasing periods of time from 1 to 4 h, with [^{35}S]methionine and cysteine added during the last hour of incubation at 25°C. Samples were processed for SDS-PAGE analysis as described above.

RESULTS

Temperature Response

Leaf tissue from *L. esculentum*, cv UC82, *L. pennellii*, and the interspecific hybrid was incubated at increasing temperatures in the presence of [^{35}S]methionine and cysteine and the proteins synthesized were analyzed on one-dimensional SDS-PAGE (Fig. 1). Tomato species, like other plants characterized to date, synthesize a complex array of proteins in response to heat shock. Several high molecular mass HSPs can be identified in cultivated tomato, 100, 93, 84, and 70 kD, and an array of low molecular mass classes, 36 and 35 kD, 25 to 21 kD, and 18 to 15 kD. Most of these classes of HSPs are present in *L. pennellii*; however, several of the small molecular mass HSPs displayed in UC82 are not present in the wild species, in particular, 23, 22.5, 16, and 15.5 kD HSPs. The HSP profile of the cultivated tomato is apparently more complex than the wild species; both genotypes are inbred, so heterozygosity is not an explanation for the difference in the number of HSPs displayed.

The interspecific F1 of two homozygous parents, would be expected to display all of the parental HSPs. Depending on the mechanisms of regulation of expression, the abundance of parental specific HSPs might be lower in the F1 since two different alleles were the source of the HSPs. We were not able to confidently assess whether the abundance of any of the parental specific HSPs were expressed in reduced amounts in the F1 using visual inspection of the autoradiograms. No

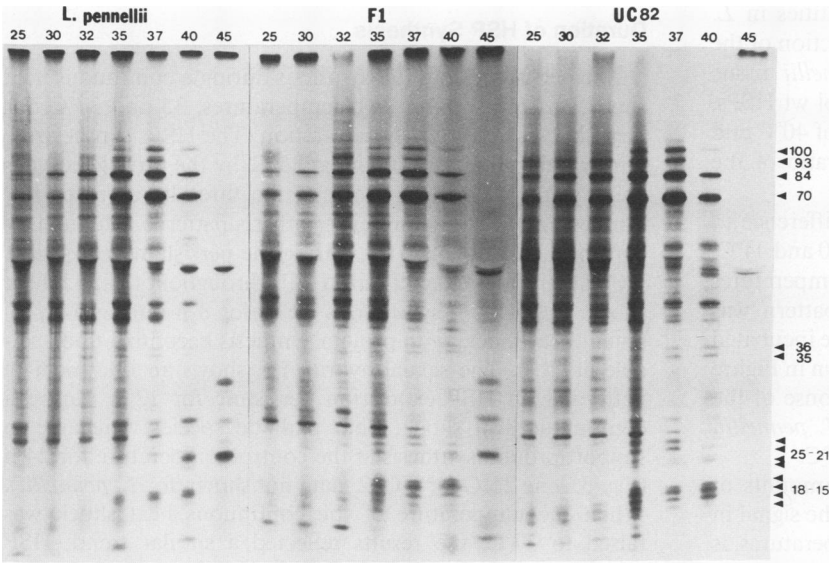

Figure 1. Effect of increasing temperature on HSP synthesis in leaves from UC82, *L. pennellii*, and F1. Leaf tissue was incubated at the indicated temperature, from 25 to 45°C, for 20 min, [^{35}S]methionine and cysteine were added, and the incubation continued for another 100 min. Samples were processed for SDS-PAGE as described in "Materials and Methods." Lanes were loaded with equal numbers of TCA precipitable counts. The major HSP classes, in kD, are indicated by arrowheads.

novel HSPs were observed to be uniquely expressed in the F1.

The temperature response of HSP synthesis was determined by incubating leaf tissue from the different genotypes, at the indicated temperatures for 2 h in the presence of [^{35}S]methionine and cysteine (Fig. 1). At 32°C, slight changes in the pattern of proteins synthesized can be detected, with the full HSP pattern becoming evident at 35°C. The temperature of maximal HSP synthesis was judged to be 37°C, based on HSP70 abundance, although the lower mol wt HSPs appear to be maximal at 35°C. By 40°C, protein synthesis has become impaired, with very little protein synthesis taking place in UC82 at 45°C. Protein synthesis persists in *L. pennellii* at 45°C, at dramatically reduced levels. All of the lanes in Figure 1 were loaded with equal amounts of precipitable cpm. The lanes in Figure 1 containing samples from 40 and 45°C heat shocked tissues have less signal. The radioactively labeled proteins in these samples, have either aggregated to a size which will not enter the stacking and/or running gel, or have degraded to small peptides which have run off the gel.

The temperature response of the sexual hybrid was the same as both parents, temperature of induction at 35°C, and temperature of maximal synthesis at 37°C.

HSP Expression at Elevated Temperatures

There was a species-specific difference in the expression of HSPs at 45°C (Fig. 1), and an analysis of the HSPs synthesized at temperatures between 40 and 44°C was undertaken in order to determine at which temperature these differences become apparent. Leaf tissue of the three genotypes was heat shocked at the indicated temperatures for 2 h in the presence of [^{35}S] methionine and cysteine, and the proteins synthesized were analyzed (Fig. 2). In addition to the HSPs whose synthesis is maximal at 37°C, marked by arrowheads, new HSPs, 47 and 22 kD, are expressed in UC82 leaf tissue at temperatures of 42°C. At 44°C, the abundance of these HSPs increases and two additional HSPs become evident, 32 and 31 kD; at this temperature all other protein synthesis is severely reduced.

The HSPs which appear at 42 and 44°C in UC82, (47, 32, 31, and 22 kD), are present in significant quantities in *L. pennellii* tissue heat shocked at 40°C. Visual inspection of the autoradiograph indicates that at 42°C, *L. pennellii* tissue synthesizes more low mol wt HSPs than high mol wt HSPs, in particular the HSPs expressed at temperatures of 40°C and greater. UC82 appears to maintain a constant ratio of the high and low mol wt HSPs.

Since there was a significant, species-specific difference in the proteins synthesized at temperatures between 40 and 44°C, the response of the F1 to very high heat shock temperatures was investigated to determine which parental pattern was displayed. The proteins synthesized in F1 leaf tissue incubated at temperatures between 40 and 44°C are also shown in Figure 2. The response of this tissue resembles the response of the UC82 parent, that is, the HSPs induced at 40°C in *L. pennellii*, and at 44°C in UC82, are induced in the F1 at 44°C.

Again, all the lanes were loaded with equal amounts of precipitable cpm, the differences in intensities of the signal in the lanes containing samples from the higher temperatures, is probably due to either aggregation or degradation of the proteins in the sample.

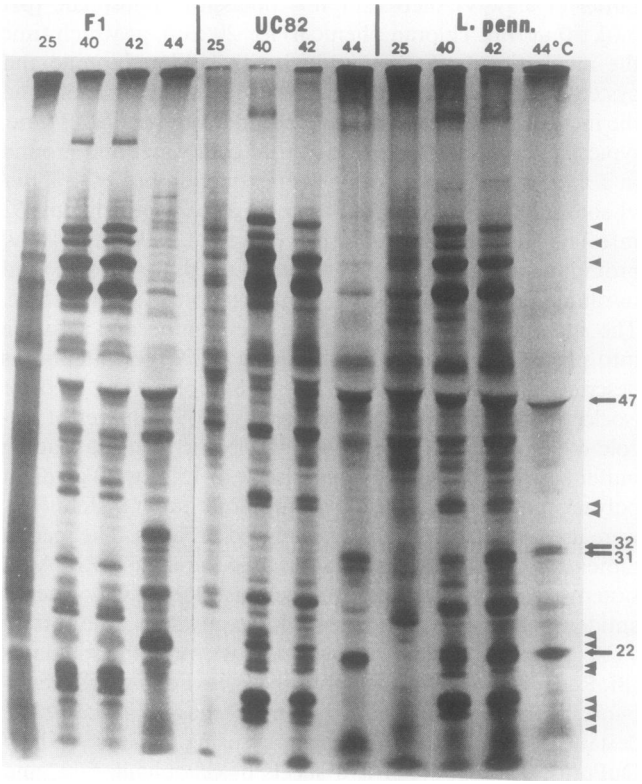

Figure 2. Effect of very high temperatures on HSP synthesis in UC82, *L. pennellii*, and F1. Leaf tissue was incubated at the indicated temperatures, 25 to 44°C, for 20 min, [^{35}S]methionine and cysteine were added, and the incubation continued for another 100 min. Samples were processed for SDS-PAGE as described in "Materials and Methods." Lanes were loaded with equal numbers of TCA precipitable counts. The major HSP classes are indicated by arrowheads, the HSPs induced only at elevated temperatures are indicated in kD with arrows.

Duration of HSP Synthesis

The persistence of HSP synthesis during a continuous heat shock was measured at two temperatures, 35 and 37°C, for heat shock of up to 24 h in duration. The HSPs synthesized, during a duration experiment at 35°C, by the three genotypes are shown in Figure 3. None of the three lines cease HSP synthesis and resume protein synthesis patterns exhibited by control incubations (25°C), during the persistent heat shock. UC82 continues to synthesize HSPs throughout the 24 h heat shock; *L. pennellii* synthesizes HSPs for 8 h during the persistent heat shock, with protein synthesis becoming undetectable at 12 h. The sexual hybrid, F1, shows an intermediate pattern, with HSP expression persisting for 12 h during a continuous heat shock. Both parental species were able to support protein synthesis at the control temperature for 24 h (Fig. 3, lane 25°C for UC82, data not shown for *L. pennellii*). When the temperature of the continuous heat shock was raised to 37°C, the results reflected a similar trend; HSP

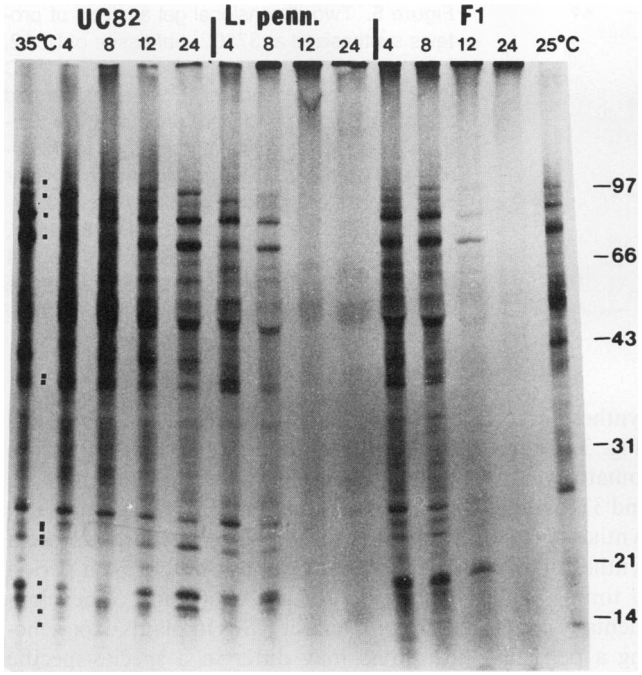

Figure 3. Duration of HSP synthesis during a persistent heat shock at 35°C. Leaf tissue of UC82, *L. pennellii*, or the F1 was incubated at 35°C for the indicated periods of time, 4 to 24 h. During the last hour of incubation, [^{35}S]methionine and cysteine were added to the samples; the samples were processed for SDS-PAGE as described in "Materials and Methods." Lanes were loaded with equal numbers of TCA precipitable counts. The lane marked 35°C contains samples of UC82 leaf tissue incubated at that temperature for 2 h in the presence of [^{35}S]methionine and cysteine; the lane marked 25°C contains samples of UC82 leaf tissue incubated at that temperature for 24 h and labeled during the last hour of incubation. The major HSP classes are marked with dots, and the migration of molecular mass size markers are indicated in kD.

synthesis was detectable in *L. pennellii* only for 4 h, in the F1 for 8 h, and in UC82 for 12 to 24 h but at greatly reduced levels (data not shown).

HSP Expression during Recovery from Heat Stress

The rates of recovery from heat shock as judged by the expression of HSPs were measured for tissue heat shocked at 40 and 42°C. The HSPs synthesized by the three genotypes following recovery from a 40°C heat shock are shown in Figure 4. During the fourth hour following a shift to control temperature of 25°C (UC82, lane 3), UC82 leaf tissue has ceased synthesis of most HSPs and resumed synthesis of 'control proteins.' This shift back to the control pattern of protein synthesis is noticeable after 2 h at the control temperature (UC82, lane 2). Heat shocked *L. pennellii* tissue has not returned to the control pattern of protein synthesis by 4 h (*L. pennellii*, lane 3). The sexual hybrid resembles the UC82 parent, in that a shift back to the control pattern of protein synthesis is apparent during the second hour after return to control temperature (F1, lane 2). When the temperature of the heat shock was elevated to 42°C, none of the genotypes returned to a control pattern of protein synthesis within the 4 h. All of the genotypes persisted in synthesizing HSPs in an undiminished fashion (data not shown).

Identification of Species-Specific HSPs

The analysis of HSPs in Figure 1, using SDS-PAGE to resolve the HSPs, could not determine if the unique parental HSPs were differentially expressed in the sexual hybrid. A more sensitive measurement of the qualitative differences in HSP expression is analysis by two-dimensional gel electrophoresis.

The HSPs synthesized by leaf tissue of the three genotypes at 37°C were resolved on two-dimensional gels (Fig. 5). Despite the fact that all of the two-dimensional gels were loaded with an equal number of precipitable counts, there is variability between the images produced. To make comparisons between the three genotypes, the most similar gel images were chosen and a common HSP was used to equate the relative abundances of the HSPs between the different samples. This HSP is indicated in Figure 5 with a plus sign.

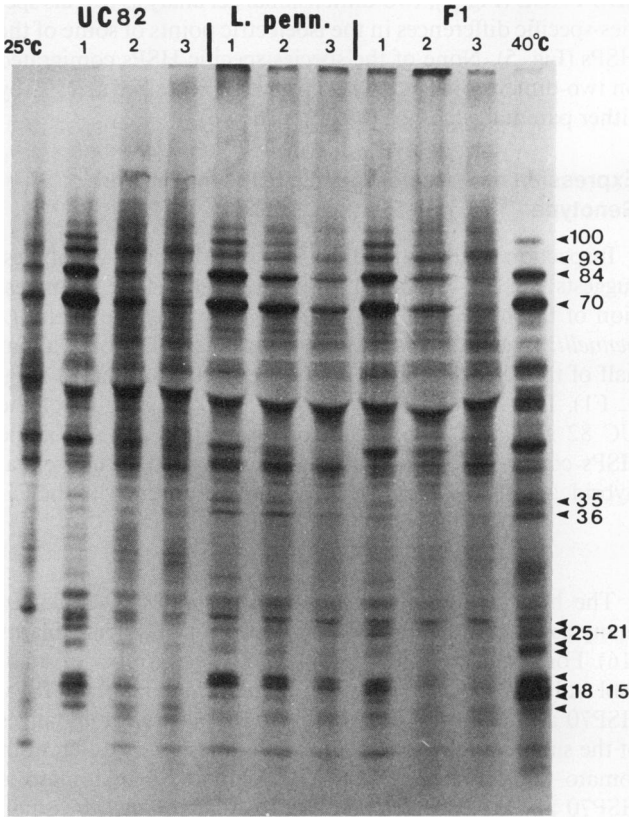

Figure 4. Recovery from HSP synthesis induced at 40°C. Leaf tissue of UC82, *L. pennellii*, and the F1 was incubated at 40°C for 2 h, and then incubated at 25°C. The samples were labeled with [^{35}S]methionine and cysteine for a 1 h period during the incubation at 25°C as follows: lanes 1, 0 to 1 h, lanes 2, 1.5 to 2.5, and lanes 3, 3 to 4 h. Lanes marked 25 and 40°C contain leaf samples of UC82 incubated at the indicated temperatures for 2 h in the presence of [^{35}S]methionine and cysteine. The sizes of the major HSP classes are indicated in kD.

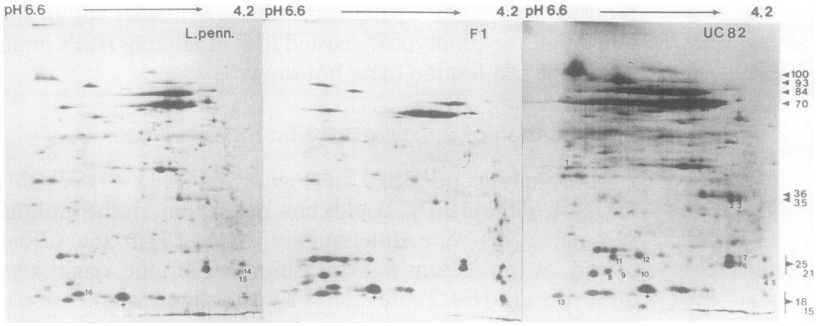

Figure 5. Two-dimensional gel analysis of proteins synthesized at 37°C. Leaf tissue of UC82, *L. pennellii*, or the F1 were incubated at 37°C for 2 h in the presence of [^{35}S]methionine and cysteine. The samples were processed for two-dimensional gel analysis as described in "Materials and Methods." The positions of the major HSP classes are indicated in kD. The species-specific HSPs are numbered in the UC82 panel, 1 through 13, and in the *L. pennellii* panel, 14 through 16. The HSP used to normalize the images is marked with a plus sign in all three panels.

The observation on SDS-PAGE that the HSP pattern of UC82 is more complex than the pattern in *L. pennellii*, is substantiated by the two-dimensional pattern. The species-specific HSPs are numbered in the respective panels of Figure 5. There are more UC82 specific HSPs, Nos. 1 to 13 in Figure 5, than there are *L. pennellii* specific HSPs, Nos. 14 to 16 in Figure 5. In addition to the species-specific differences with respect to size of the low mol wt range which were evident on SDS-PAGE (Fig. 1), two-dimensional gel analysis reveals species-specific differences in the isoelectric points of some of the HSPs (Fig. 5). None of the species-specific HSPs comigrated on two-dimensional gels with proteins synthesized at 25°C by either parental genotype (data not shown).

Expression of Species-Specific HSPs in a Hybrid Genotype

Inspection of the patterns of HSPs in the three genotypes, suggests that the sexual hybrid is not intermediate in expression of the parental HSP patterns. While all three of the *L. pennellii* specific HSPs are expressed in the F1, only about half of the UC82 specific HSPs are expressed in the F1 (Fig. 5, F1). In particular, Nos. 1 to 3, 6, 7, 11, and 13 of the UC 82 specific HSPs are not present in the F1. All of the HSPs common to both parents are expressed in the sexual hybrid, and there were no HSPs expressed uniquely in the F1.

DISCUSSION

The heat shock response of tomato species is similar in many aspects to the response described for other crop plants (16). For instance, tomato produces a large number of small mol wt HSPs, in addition to the ubiquitous high mol wt HSPs, HSP70 and HSP84 (Figs. 1 and 5). The relative abundances of the small and high mol wt proteins are slightly different in tomato than in other crops. The major HSP in tomato is HSP70 and its cognates (Fig. 5). In other plants, the small mol wt HSPs appear to be the most prominent species (9). During heat shock, at temperatures above 35°C, in tomato, like other plants, very few proteins other than those induced by heat are detected following *in vivo* labeling (Fig. 1). However, the heat shock response of tomato differs from that of corn or soybean on several points. In soybean, after 4 to 8 h of continuous heat shock, the abundance of HSPs diminishes, and developmentally controlled proteins begin to reappear in samples (9). Persistent heat shock induces persistent HSP synthesis in tomato, until no protein synthesis can be detected (Fig. 3). In our analysis of the duration of HSP synthesis in tomato, we investigated the response at two temperatures, 35 and 37°C, the temperatures of induction and of maximal HSP synthesis. We did not observe a return to control protein synthesis patterns at either temperature within a 24 h period of time. Our results indicate that the expression of developmentally controlled proteins and of HSPs in plants experiencing a persistent heat stress may differ in a species-specific manner.

In addition to the induction of typical HSPs at temperatures approximately 10°C higher than standard growth temperatures, an additional set of heat induced proteins were observed at temperatures 15 to 20°C higher than standard growth temperatures (Fig. 2). These proteins could be new gene products which are induced at the higher temperatures in a manner equivalent to the induction of the classical HSPs. Alternatively, these proteins could be degradation or proteolytic products of the classical HSPs. Evidence in support of this interpretation is the observation that for UC82 and the F1, the abundance of some of the classical HSPs appears to diminish in samples with significant quantities of the high temperature HSPs. However, there is evidence which suggests that these high temperature HSPs are new gene products: (a) All three genotypes produce the same size classes of high temperature HSPs, and (b) *L. pennellii* produces these high temperature HSPs under conditions where the abundance of the classical HSPs seems unchanged.

When the inheritance of quantitative traits in the F1 generation of crosses between *L. esculentum* and *L. pennellii* is measured, several of these characters do not show an expected intermediate value between the two parental values. The F1 generation is skewed toward either the *L. pennellii* parent, or the *L. esculentum* parent for many traits depending on the character (24, 28, 30). However, when the inheritance of biochemical markers, *i.e.* isozymes, is measured in the F1 generation, both parental alleles are usually expressed (26). In our analysis of the heat shock response in the interspecific hybrid, we did not establish whether any of the species-specific HSPs were alleles; however, it is clear that the F1 does not display both sets of parental patterns of HSPs. The F1 generation displayed all of the HSPs unique to the *L. pennellii* parent, and many but not all of the HSPs unique to the *L. esculentum* parent.

There are several possibilities which could explain the ab-

sence of some of the *L. esculentum* specific HSPs. Some of the HSPs may have an altered mobility on two-dimensional gels when expressed in a hybrid nuclear background. Perhaps alterations in posttranslational processing or organelle targeting are modified in the presence of activities contributed by the *L. pennellii* genome. We consider this possibility unlikely since the maternal parent in the construction of the F1 was *L. esculentum*, and therefore the organelles would be from the *L. esculentum* parent. Furthermore, if posttranslational processing reactions were modified in the hybrid background, one could expect to see F1 specific HSPs and none were observed.

An alternative explanation is that the missing HSPs could be recessive alleles. In that case an analysis of the F2 generation might indicate which HSPs were allelic. Preliminary results towards this goal have ruled out the possibility that the missing HSPs are recessive alleles, since individuals in the F2 generation have been obtained which express all 16 of the species-specific HSPs.

A third explanation, for the missing HSPs in the F1, is that the regulation of some of the HSPs is coordinated, and the gene(s) responsible for that coordinated regulation is recessive to the *L. pennellii* allele. In this case, one would expect to obtain individuals in the F2 generation which are homozygous for the *L. esculentum* allele for this regulatory gene and therefore express all of the species-specific HSPs. The results observed in the heat shock response at temperatures above 40°C produced a similar type of result. The HSP pattern of expression of a suite of HSPs in the F1 was not intermediate between the two parental responses, but displayed only the *L. esculentum* parental pattern (Fig. 2). In this case the *L. esculentum* pattern was dominant.

In some aspects of the heat shock response, the F1 generation displayed an intermediate response. In both duration and recovery assays, the parental species differed in the length of time to recover from heat shock and in their ability to continue protein synthesis, the F1 generation displayed times intermediate to the two parental responses (Figs. 3 and 4). We did not determine which step in transcription or translation was differentially heat sensitive in the two species. Crudely, our results suggest that the overall performance of the hybrid genotype is an average of the mixed population of differentially heat sensitive enzymes, protein and RNA species contributed by the two parental genomes.

The heat shock response of plants is expected to play an adaptive role, allowing the plant to develop thermotolerance. The evidence linking HSP expression and acquired thermotolerance in plants is substantial (12), but debatable (13, 14). However, the role of HSP expression in heritable thermotolerance is still quite speculative. While there have been several reports in the literature correlating differences in HSP patterns and differences in heritable thermotolerance (10, 23), the genetic proof linking thermotolerance and specific alterations in HSP expression is still lacking. In one case, when the appropriate genetic lines of cotton were compared, differences in HSP expression observed between thermotolerant and thermosensitive lines were not found to be linked with the thermotolerance phenotype (7).

The two tomato species analyzed in this study differ in drought tolerance (25), in water use efficiency (18) and in salt tolerance (29), with *L. pennellii* in all cases being the more tolerant or efficient genotype. Differences in thermal tolerance have not been described for these two species, and in preliminary studies we did not detect any differences in seed germination rates at elevated temperatures (data not shown). One of the mechanisms of drought tolerance used by *L. pennellii* is a remarkable leaf water retention rate (25). A predicted consequence of this mechanism would be that leaf temperatures of *L. pennellii* should be higher than leaf temperatures of *L. esculentum* at a given air temperature. We have observed in our greenhouse, where the air temperatures range from 25 to 36°C, the leaf temperatures of UC82, the F1 and *L. pennellii*, averaged 3.1, 2.4, and 1.7°C below air temperature, respectively. While these observations do not demonstrate differences in thermal tolerance between these genotypes, they do suggest that in its native arid environment *L. pennellii* may experience thermal stress.

Under prolonged laboratory heat shock conditions (Fig. 3). *L. pennellii* was unable to persist in protein synthesis as long as UC82. Similarly, in recovery experiments, UC82 returned to 'control' protein synthesis patterns faster than *L. pennellii* (Fig. 4). These results suggest that positive HSP expression is not a likely component in adaptation of *L. pennellii* to conditions which cause elevated leaf temperatures.

In summary, a comparison of the heat shock response in two sexually compatible species of tomato, *L. esculentum* and *L. pennellii*, revealed a number of species-specific differences with regard to unique HSPs, HSPs expressed at very high temperatures, the regulation of the duration of protein synthesis during heat shock, and the rate of recovery from HSP synthesis following return to control temperature. These differences in parental responses to heat shock were differentially expressed in the F1, in some cases an intermediate response was displayed in the F1: duration and recovery responses, and expression of many of the species-specific HSPs. In other instances only one parental response was displayed in the F1: the pattern of HSPs expressed at very high temperatures, and the absence of many of the *L. esculentum* specific HSPs.

ACKNOWLEDGMENT

We would like to thank Petoseed and Dr. Charles Rick, University of California, Davis for generously supplying us with tomato seed. We would like to thank Dr. Elizabeth Vierling for her critical comments and advice on this project.

LITERATURE CITED

1. **Adams LD** (1987) Two dimensional gel electrophoresis using the ISO-DALT system. *In* FM Ausubel, R Brent, RE Kingston, DD Moore, JG Seidman, JA Smith, K Struhl, eds, Current Protocols in Molecular Biology. John Wiley & Sons, New York, pp 10.3.1–10.3.12
2. **Altschuler M, Mascarenhas JP** (1982) Heat shock proteins and effects of heat shock in plants. Plant Mol Biol **1:** 103–115
3. **Baszczynski CL, Walden DB, Atkinson BG** (1982) Regulation of gene expression in corn (*Zea mays* L.) by heat shock. Can J Biochem **60:** 569–579
4. **Baszczynski CL, Walden DB, Atkinson BG** (1985) Maize genome response to thermal shifts. *In* BG Atkinson, DB Walden, eds,

Changes in Eukaryotic Gene Expression in Response to Environmental Stress. Academic Press, Orlando, pp 349–371
5. **Burke JJ, Hatfield JL, Klein RR, Mullet JE** (1985) Accumulation of heat shock proteins in field grown cotton. Plant Physiol **78:** 394–398
6. **Duck N, McCormick S, Winter J** (1989) Heat shock protein hsp70 cognate gene expression in vegetative and reproductive organs of *Lycopersicon esculentum*. Proc Natl Acad Sci USA **86:** 3674–3678
7. **Fender SE, O'Connell, MA** (1989) Heat shock protein expression in thermotolerant and thermosensitive lines of cotton. Plant Cell Rep **8:** 37–40
8. **Key JL, Lin C-Y, Chen YM** (1981) Heat shock proteins of higher plants. Proc Natl Acad Sci USA **78:** 3526–3530
9. **Key JL, Kimpel J, Vierling E, Lin C-Y, Nagao RT, Czarnecka E, Schoffl F** (1985) Physiological and molecular analyses of the heat shock response in plants. *In* BG Atkinson, DB Walden, eds, Changes in Eukaryotic Gene Expression in Response to Environmental Stress. Academic Press, Orlando, pp 327–348
10. **Krishnan M, Nguyen HT, Burke JJ** (1989) Heat shock protein synthesis and thermal tolerance in wheat. Plant Physiol **90:** 140–145
11. **Laemmli UK** (1970) Cleavage of the structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685
12. **Lin CY, Roberts JK, Key JL** (1984) Acquisition of thermotolerance in soybean seedlings. Plant Physiol **74:** 152–160
13. **Lindquist S** (1986) The heat shock response. Annu Rev Biochem **55:** 1151–1191
14. **Lindquist S, Craig EA** (1988) The heat shock proteins. Annu Rev Genet **22:** 631–677
15. **Mans RJ, Novelli GD** (1960) Measurement of the incorporation of radioactive amino acids into protein by a filter paper disk method. Arch Biochem Biophys **94:** 48–53
16. **Mansfield MA, Key JL** (1987) Synthesis of the low molecular weight heat shock proteins in plants. Plant Physiol **84:** 1007–1017
17. **Marmiroli N, Lorenzoni C, Stanca AM, Terzi V** (1989) Preliminary study of the inheritance of temperature stress proteins in barley (*Hordeum vulgare* L.) Plant Sci **62:** 147–156
18. **Martin B, Thorstenson YR** (1988) Stable carbon isotope composition ($\delta^{13}C$), water use efficiency and biomass productivity of *Lycopersicon esculentum*, *Lycopersicon pennellii* and the F1 hybrid. Plant Physiol **88:** 213–217
19. **Nover L, Scharf KD** (1984) Synthesis, modification and structural binding of heat-shock proteins in tomato cell cultures. Eur J Biochem **139:** 303–313
20. **Nover L, Scharf KD, Neumann D** (1983) Formation of cytoplasmic heat shock granules in tomato cell cultures and leaves. Mol Cell Biol **3:** 1648–1655
21. **O'Connell MA, Hanson MR** (1985) Somatic hybridization between *Lycopersicon esculentum* and *Lycopersicon pennellii*. Theor Appl Genet **70:** 1–12
22. **O'Connell MA, Hanson MR** (1987) Regeneration of somatic hybrids formed between *Lycopersicon esculentum* and *L. pennellii*. Theor Appl Genet **75:** 83–89
23. **Ougham HJ, Stoddart JL** (1986) Synthesis of heat shock proteins and acquisition of thermotolerance in high temperature tolerant and high temperature susceptible lines of *Sorghum*. Plant Sci **44:** 163–167
24. **Rick CM** (1960) Hybridization between *Lycopersicon esculentum* and *Solanum pennellii*: phylogenetic and cytogenetic significance. Proc Natl Acad Sci USA **16:** 78–82
25. **Rick CM** (1973) Potential genetic resources in tomato species: clues from observations in native habitats. *In* AM Srb, ed, Genes Enzymes and Populations, Vol 2. Plenum Press, New York, pp 255–269
26. **Rick CM** (1983) Tomato. *In* SD Tanksley, TJ Orton, eds, Isozymes in Plant Genetics and Breeding, Part B. Elsevier, Amsterdam, pp 147–166
27. **Scharf KD, Nover L** (1982) Heat-shock-induced alterations of ribosomal protein phosphorylation in plant cell cultures. Cell **30:** 427–437
28. **Tal M** (1967) Genetic differentiation and stability of some characters that distinguish *Lycopersicon esculentum* Mill. from *Solanum pennellii* Cor. Evolution **21:** 316–333
29. **Tal M, Shannon MC** (1983) Salt tolerance in the wild relatives of the cultivated tomato: responses of *L. esculentum*, *L. cheesmanii*, *L. peruvianum*, *Solanum pennellii* and the F1 hybrids to high salinity. Aust J Plant Physiol **10:** 109–117
30. **Tanksley SD, Medina-Filho H, Rick CM** (1982) Use of naturally occurring enzyme variation to detect and map genes controlling quantitative traits in an interspecific backcross of tomato. Heredity **49:** 11–25