Plant Physiol. (1984) 75, 778–780
0032-0889/84/75/0778/03/$01.00/0

Heat Stress Responses in Cultured Plant Cells[1]

HEAT TOLERANCE INDUCED BY HEAT SHOCK *VERSUS* ELEVATED GROWING TEMPERATURE

Received for publication December 2, 1983 and in revised form March 27, 1984

MIN-TZE WU AND STEPHEN J. WALLNER*
Department of Horticulture, Colorado State University, Fort Collins, Colorado 80523

ABSTRACT

Using cultured pear (*Pyrus communis* cv Bartlett) cells, heat tolerance induced by heat shock was compared to that developed during growth at high temperature. After growth at 22°C, cells exposed to 38°C for 20 minutes (heat shock) showed maximum increased tolerance within 6 hours. Cells grown at 30°C developed maximum heat tolerance after 5 to 6 days; this maximum was well below that induced by heat shock. Heat shock-induced tolerance was fully retained at 22°C for 2 days and was only partly lost after 4 days. However, pear cells acclimated at 30°C lost all acquired heat tolerance 1 to 2 days after transfer to 22°C. In addition, cells which had been heat-acclimated by growth at 30°C showed an additional increase in heat tolerance in response to 39°C heat shock. The most striking difference between heat shock and high growth temperature effects on heat tolerance was revealed when tolerance was determined using viability tests based on different cell functions. Growth at 30°C produced a general hardening, *i.e.* increased heat tolerance was observed with all three viability tests. In contrast, significantly increased tolerance of heat-shocked cells was observed only with the culture regrowth test. The two types of treatment evoke different mechanisms of heat acclimation.

Heat shock, *i.e.* brief exposure to supraoptimal temperature, alters gene expression and leads to increased heat tolerance in a wide range of organisms (10). However, there is no direct evidence that synthesis of unique sets of proteins (HS[2] proteins) is causally related to heat acclimation. Minton *et al.* (9) theorized that HS proteins protect the cell from heat injury by stabilizing other proteins in a nonspecific manner. Effective testing of this and other models would be facilitated by convenient sources of HS proteins in large quantities. Since plant cell suspensions may be useful in this regard, we have examined HS responses of pear cells in liquid culture. We also compared HS effects to those observed during continuous exposure to 30°C. Elevated growing temperatures induce heat hardening in many plants, but how this compares to HS-induced heat tolerance is not known. Some reviews of plant response to high temperature have implied that HS and longer term elevated growth temperatures are comparable in their effect on heat tolerance (1, 12). The equation $T = a - b \log Z$(6), used to describe the relationship between heat stress temperature (T) and time (Z), also suggests that the response to heat is similar over a wide range of temperatures (since a and b are constants). If so, then heat acclimation induced by brief HS and that induced by prolonged but more moderate heat (*e.g.* 30°C) would have similar mechanisms. However, results presented in this paper indicate that HS and prolonged exposure to 30°C increase the heat tolerance of pear cells in clearly different ways.

[1] Supported in part by a grant from the Faculty Council Committee on Research, Colorado State University. Published with the approval of the Colorado Agricultural Experiment Station as Scientific Series Paper No. 2908.

[2] Abbreviations: HS, heat shock; TTC, triphenyl-tetrazolium chloride.

MATERIALS AND METHODS

All experiments were conducted with suspension-cultured cells of pear (*Pyrus communis* cv Bartlett) which were used in our previous studies (13, 14). The culture medium and most methods (culture maintenance, growth conditions and measurement, heat stress treatment, and viability tests) were as described before (13). All stock cultures and experimental controls were grown at 22°C.

Heat shock was administered to cells from 7-d-old suspension cultures using 5-ml aliquots in 1 × 10 cm test tubes. Heat treatment was accomplished in a water bath at the HS temperature; temperature equilibration in the 5-ml aliquots occurred within 3 min. The HS treatments lasted 20 min after which six of the 5-ml heat-shocked cell suspensions were transferred to empty, sterile 125-ml Erlenmeyer flasks and maintained at 22°C with shaking. Non-heat-shocked controls were subjected to the same sample handling procedures.

RESULTS AND DISCUSSION

HS-Induced Tolerance. Pear cells exposed to 38°C for 20 min and then incubated for 24 h at 22°C showed greatly increased tolerance of a subsequent heat stress treatment (Fig. 1). The 38°C heat-shocked cells produced nearly the same (92%) dry weight gain in the 10 d following a 43°C, 20-min stress as did their nonstressed controls. The 36°C HS resulted in considerably less heat tolerance than did 38°C HS. It is interesting that although 20 min at 40°C initiated changes leading to heat tolerance, 85 min at 40°C or 20 min at 42°C killed the cultures (13). The 38°C optimum for HS-induced heat tolerance of cultured pear cells is close to the optimum for HS protein synthesis in other organisms. Barnett *et al.* (3) reported that 39°C was optimal for HS protein synthesis in cultured tobacco and soybean cells. Temperature optima for HS responses have not been precisely identified in most cases, but 36°C to 40°C has been found effective for HS protein synthesis in maize roots (4), soybean hypocotyls (5), and *Drosophila* cells (2), and for development of heat tolerance in soybean seedlings (7) and yeast cells (8). Correlations consistent with a causal relationship between HS protein synthesis and acquired heat tolerance have been described for yeast cells (8) and soybean seedlings (7). Schroeder (11) showed that heat tolerance was induced in avocado tissue cultures following 10 to 30 min HS. However, the lowest temperature he used was 45°C, which is above the optimum for HS protein synthesis in cultured tobacco and soybean cells (3). Our data (Fig. 1) indicate that HS-induced heat tolerance in suspension-cultured plant cells is op-

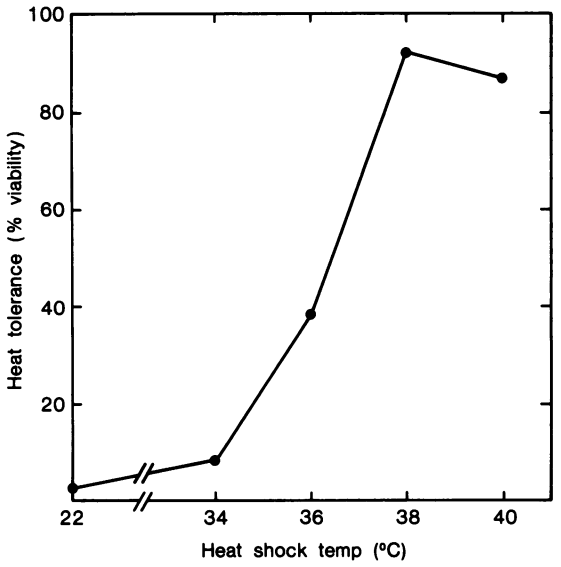

FIG. 1. Effect of heat shock on the heat tolerance of cultured pear cells. Cell suspensions were exposed to the indicated temperatures for 20 min and then incubated at 22°C for 24 h. Heat tolerance was then determined by exposing cells to a 43°C, 20-min stress and measuring regrowth capacity; growth was measured after 10 d at 22°C.

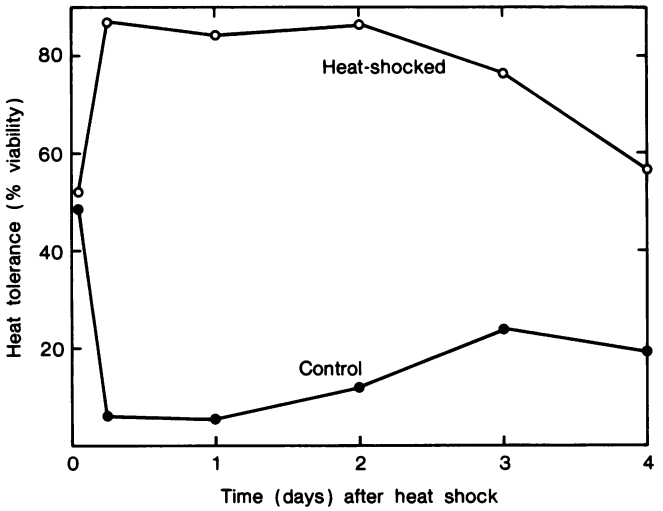

FIG. 2. Time course (at 22°C) of heat tolerance changes in heat-shocked and control pear cells. Heat shock was 38°C for 20 min. Heat tolerance was determined by exposing cells to a 43°C, 20-min stress (given at various times after the initial HS) and measuring regrowth capacity; growth was measured after 10 d at 22°C.

timal at 38°C, a temperature which initiates near maximal HS protein synthesis in almost all reported studies.

The increase in pear cell heat tolerance was completed within 6 h following the 20-min inductive HS (Fig. 2). The heat tolerance of heat-shocked cells was much greater than that of controls during the days following HS (Fig. 2). As discussed previously (14), the apparent decline in control cell heat tolerance during the 1st d (Fig. 2) probably reflects loss of a transient tolerance induced by culture handling, *i.e.*, return to a normal condition. In the experiments for Figure 2, zero time measurements could not be obtained. However, without the handling involved in HS treatment, 22°C-grown cells showed less than 15% survival of a 43°C, 20-min stress (13). Heat tolerance was fully maintained for 2 d at 22°C and then began to gradually decline (Fig. 2). The 2 to 3 d persistence of heat tolerance in pear cells agrees well with limited reports for intact plants. HS-induced tolerance was fully maintained in cabbage leaves for 2 d (12) and in bean leaves for at least 3 d (15). If HS protein confer heat tolerance directly, they must be relatively stable because their synthesis has usually been found to cease soon after return to normal temperature (3–5).

Heat Tolerance Induced at 30°C. Pear cells in suspension cultures grown at 30°C began to show increased heat tolerance after 3 d (Fig. 3) and reached maximum tolerance at 6 d. This is clearly different from the tolerance induced by 38°C (Figs. 1 and 2) which required only a 20-min heat treatment. The maximum tolerance achieved by cells grown at 30°C was between 60 and 65 (% viability after a 43°C, 20-min stress, based on the regrowth test) while that induced by 38°C HS was between 90 and 95 (Figs. 1 and 2).

Differences between HS and 30°C-Induced Tolerance. In addition to the difference in maximum heat tolerance attained, cells induced by HS and those hardened at 30°C did not lose heat tolerance in the same way upon return to 22°C (Fig. 4). Unlike heat-shocked cells, those grown at 30°C lost all acquired heat tolerance between the 1st and 2nd d. The heat-shocked cells retained full tolerance for 2 d and were still considerably more tolerant than controls after 4 d at 22°C (Fig. 2). Declining heat tolerance in a suspension culture could be due to loss of the acquired physiological condition in existing cells and/or to growth, *i.e.* the production of new, non-hardy cells at 22°C. However, cultures grown at 22°C for 7 d (*i.e.* those used in HS experiments) and at 30°C for 6 d were in the same phase of culture development (data not shown), so their growth potential (nutrients remaining in the medium) was probably similar.

Another observation which suggests that acclimation by HS and by growth at 30°C are fundamentally different is that HS greatly increased the tolerance of the 30°C-grown cells. Because the initial level of tolerance was higher than for 22°C-grown cells (Fig. 3), higher HS (39°C) and heat stress (45°C) temperatures were used to study the HS response of 30°C-grown cells. The HS response was comparable to that obtained with 22°C-grown cells. Following a 39°C HS, the 30°C-grown cells rapidly developed tolerance which allowed nearly complete survival of a subsequent 45°C stress. As in our other experiments (Fig. 2; Ref. 14), control (non-HS) cells received the same handling (pipetting) treatment as did the heat-shocked cells and responded to this handling with a rapid, transient rise in tolerance (data not shown). As a result, the most appropriate comparison of heat tolerance is 24 h after

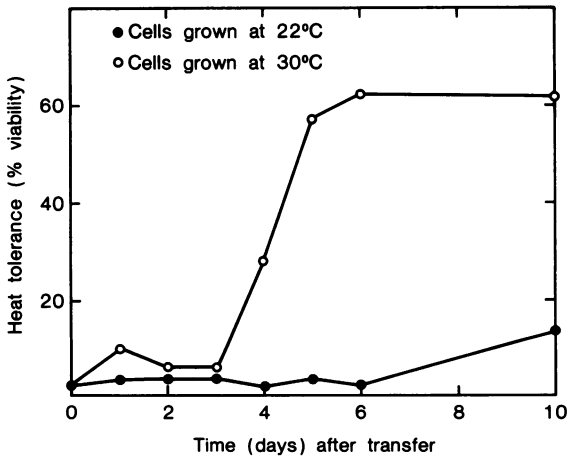

FIG. 3. Development of heat tolerance in cultured pear cells grown at 30°C. Cell suspensions were initiated from 22°C-grown cultures and half were placed immediately at 30°C for periods up to 10 d. Heat tolerance was determined by exposing cells to a 43°C, 20-min stress and measuring regrowth capacity; growth was measured after 10 d at 22°C.

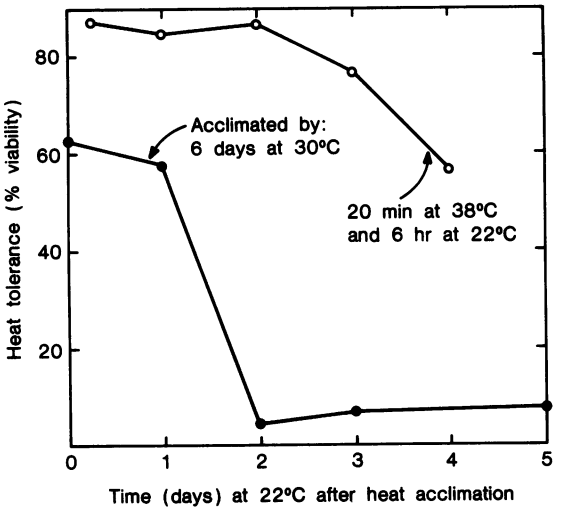

FIG. 4. Loss of heat tolerance in heat-acclimated pear cells during incubation at 22°C. Cells were acclimated by heat shock (38°C, 20 min) or elevated growing temperature (30°C, 6 d). Heat tolerance was determined by exposing cells to a 43°C, 20-min stress and measuring regrowth capacity; growth was measured after 10 d at 22°C.

Table I. *Comparison of Heat Shock and Elevated Growing Temperature Effects on the Heat Tolerance of Pear Cells as Measured with Three Different Viability Tests*

For HS, cells were exposed to 38°C for 20 min and then incubated at 22°C for 24 h. For the elevated growing temperature treatment freshly transferred cells were grown at 30°C for 6 d. Following heat acclimation by HS or elevated growing temperature, cells were heat stressed and injury measurements compared to those obtained with control cells (grown at 22°C).

Viablity Test	Stress Conditions	Increase in Heat Tolerance (Injury to Control + Injury to Acclimated) in Response to:	
		6 d at 30°C	HS (20 min, 38°C)
TTC reduction	52°C, 20 min	4.5	1.3
Elecrolyte leakage	48°C, 40 min	2.6	1.1
Culture regrowth	43°C, 20 min	3.0	11.7

HS; at this time, 30°C-grown control and heat-shocked cells showed 9% and 81% survival, respectively, of the 45°C stress. A HS-induced increase in heat tolerance of cells hardened at 30°C does not necessarily mean that the initial hardiness was induced by a different mechanism. However, in this case the percent increase in tolerance induced by HS was nearly as great as that induced by HS treatment of 22°C-grown cells (Fig. 2). If the heat hardening during 8 d at 30°C was similar to HS, the subsequent HS response would likely have been less striking.

The most important difference between the HS and 30°C response of pear cells is summarized in Table I. These data show effects on heat tolerance as measured with three viability tests. These tests (electrolyte leakage, regrowth, and TTC reduction) are commonly used to assess stress injury (6) and were previously characterized as indices of pear cell heat injury (13). Acclimation during growth at 30°C lead to a general increase in the heat tolerance of all measured cellular functions. The capacity for TTC reduction and culture regrowth potential after heat stress were increased 4.5- and 3-fold, respectively. Resistance to membrane injury (indicated by electrolyte leakage) was also substantially increased by growth at 30°C. In contrast to the general heat hardening developed at 30°C, significant HS-induced tolerance was detected only with the regrowth viability test. The capacity for post-stress culture growth was greatly enhanced by HS (Figs. 1 and 2; Table I), but heat-shocked cells were not resistant to the direct injuries which TTC reduction and electrolyte leakage tests measure (Table I).

Recognition of differences between HS and elevated growth temperature effects on heat tolerance is critical to the study of plant performance at high temperature. We conclude that heat acclimation in response to HS and to growth at 30°C occur via different mechanisms. That is, $T = a - b \log Z$ (6) does not describe the relationship between time and temperature for heat acclimation over the entire range of supraoptimal temperatures. The threshold for HS appears to be between 30°C and 35°C for most cells, and this agrees with our results (Fig. 1). Key *et al.* (5) grew their control soybean seedlings at 28°C to 30°C, and maize roots showed little or no HS protein synthesis at 30°C, but substantial synthesis at 35°C (4). However, beans developed tolerance after 20 s at 50°C (15), 2 to 4 min at 45°C (Key, personal communication), and 10 to 20 min at 40°C (7). Thus, between its threshold and maximum temperatures, HS may elicit adaptive responses which are as dependent on a time-temperature interaction as is the development of heat injury (6).

The pear cells used in this study grow as a well-separated suspension, so they are easy to manipulate and treat. The cells developed heat tolerance in response to treatments known to induce HS protein synthesis in a manner different from the response to elevated growth temperature. Thus, pear suspension cultures may provide a useful system for further, more detailed study of HS and other temperature-related phenomena.

LITERATURE CITED

1. ALEXANDROV V, AG LOMAGIN, NL FELDMAN 1970 The responsive increase in thermostability of plant cells. Protoplasma 69: 417–458
2. ASHBURNER M, JJ BONNER 1979 The induction of gene activity in drosophila by heat shock. Cell 17: 241–254
3. BARNETT T, M ALTSCHULER, CN MCDANIEL, JP MASCARENHAS 1980 Heat shock induced proteins in plant cells. Dev Genet 1: 331–340
4. COOPER PT, HD HO 1983 Heat shock patients in maize. Plant Physiol 71: 215–222
5. KEY JL, CY LIN, YM CHEN 1981 Heat shock proteins of higher plants. Proc Natl Acad Sci USA 78: 3526–3530
6. LEVITT J 1980 Responses of plants to environmental stresses. Volume I Chilling, Freezing and High Temperature Stress. Academic Press, New York
7. LIN C-Y, JK ROBERTS, JL KEY 1984 Acquisition of thermotolerance in soybean seedlings: synthesis and accumulation of heat shock proteins and their cellular localization. Plant Physiol 74: 152–160
8. MCALISTER L, DB FINKELSTEIN 1980 Heat shock proteins and thermal resistance in yeast. Biochem Biophys Res Commun 93: 819–824
9. MINTON KW, KARMIN, GM HAHN, AP MINTON 1982 Nonspecific stabilization of stress-susceptible proteins by stress-resistant proteins: A model for the biological role of heat shock proteins. Proc Natl Acad Sci USA 79: 7107–7111
10. SCHLESINGER MJ, G ALIPERTI, PM KELLEY 1982 The response of cells to heat shock. Trends Biochem Sci 4: 221–225
11. SCHROEDER CA 1963 Induced temperature tolerance of plant tissue *in vitro*. Nature 200: 1301–1302
12. SULLIVAN CY, NV NORCIO, JD EASTIN 1977 Plant responses to high temperatures. *In* A Muhammed, R Aksel, RC von Borstel, eds, Genetic Diversity in Plants. Plenum Press, New York, pp 301–317
13. WU MT, SJ WALLNER 1983 Heat stress responses in cultured plant cells. Development and comparison of viability tests. Plant Physiol 72: 817–820
14. WU MT, SJ WALLNER, JW WADDELL 1984 Heat stress responses in cultured plant cells. Effect of culture age and handling. Plant Physiol 74: 944–946
15. YARWOOD CE 1961 Acquired tolerance of leaves to heat. Science 134: 941–942