Thermotolerance Is Developmentally Dependent in Germinating Wheat Seed

Rollin H. Abernethy *, David S. Thiel, Nancy S. Petersen, and Kenneth Helm

Department of Plant, Soil and Insect Sciences (R.H.A., D.S.T., K.J.H.), and Department of Molecular Biology (N.S.P.), University of Wyoming, Laramie, Wyoming 82071

ABSTRACT

During the initial 9 to 12 hours of imbibition, the imbibing wheat (*Triticum aestivum* L.) seed was found to exhibit substantial tolerance to high temperature relative to later times of imbibition. Tolerance was assessed by seed viability and seedling growth. This initial high temperature tolerance gradually declines with increasing time of seed imbibition. A range of 2 hour heat pretreatments (38–42°C) prior to imposition of a 2 hour heat shock (51–53°C) during this same 9 to 12 hour interval was unable to increase survival or seedling growth over that of seed that did not receive a pretreatment. However, after 9 to 12 hours of imbibition the pretreatment provided both increased survival and increased seedling growth, measured 120 hours later, i.e., classical thermotolerance could be acquired. This response is called a ‘thermotolerance transition.’ Isolated embryos responded in a similar manner using a 2,3,5-triphenyltetrazolium chloride assay for viability determination following heat treatments. The high temperature tolerance during early imbibition indicates that the thermotolerance transition involves the loss of an existing thermotolerance coincident with acquiring the ability to become thermotolerant following heat pretreatment. Despite the inability to acquire thermotolerance, heat shock protein synthesis was induced by heat shock immediately upon imbibition of wheat seed or isolated embryos. Developmentally regulated heat shock proteins of 58 to 60, 46, 40, and 14 kilodaltons were detected at 1.5 hours of imbibition following heat shock, but were absent or greatly reduced by 12 hours. Constitutive synthesis of 70 and 90 kilodalton hsp groups appeared to be greater at 1.5 hours of imbibition than at 12 hours of imbibition.

Thermotolerance is defined as the ability of an organism to survive normally lethal temperatures with prior exposure to a mild heat stress (6, 8, 13, 18). Although the mechanism for acquiring thermotolerance is unclear, heat stress is known to induce synthesis of heat shock proteins.

Substantial correlative evidence for hs\(^+\) protein involvement in adaptation to stress has been presented for a broad range of organisms (13, 14, 17, 18). Evidence for hs protein's role in the acquisition of thermotolerance in *Drosophila*, *Xenopus*, and other animals is found during embryonic developmental stages. There are specific stages in which hs protein induction cannot be obtained with long duration of (15 to 60 min) hs. These stages coincide with extreme sensitivity to heat stress (7, 9). Similarly, in a number of organisms, the induction and decay of hs proteins correlates with the ability or inability to survive hyperthermic stress (14). The high degree of evolutionary conservation of heat shock genes and the hs response (27) has further strengthened the argument in support of hs protein's role in stress protection. Recently, however, it has been shown that not all the hs proteins are required for thermal protection in *Drosophila* and yeast (28), and antibiotic treatments which initially inhibit protein synthesis can provide thermal protection in *Tetrahymena* (8). The function of hs proteins in thermotolerance has remained elusive.

Although the eucaryotic hs response has been more extensively studied in *Drosophila*, yeast and mammalian cells, current evidence indicates the response in higher plants is similar (2, 5, 11, 22). Plant hs-induced proteins differ from those of other organisms in that there is a greater abundance of low M₅ (15–27 kD) proteins. Also, normal protein synthesis is repressed to a lesser degree by hs (11). The induction of hs protein synthesis is observed in virtually all vegetative tissues of higher plants and in the embryo during its development (5, 16). Heat shock protein synthesis is induced by a sudden increase in temperature of 8° to 10°C over the current growth temperature, by a gradual temperature increase of greater magnitude, by several heavy metal ions, and by other stresses to the organism (11). Lin et al. (13) reported that germinated soybean seedlings survive a 2 h, 45°C hs if first provided one of several stresses: a 40°C hs, brief (10 min) 45°C hs, or 50 \( \mu \)M arsenite pretreatments, all of which were shown to also induce hs protein synthesis.

Whereas previous reports of plant thermotolerance utilized seedlings or vegetative plant tissues (13), the present study focuses on early germination events. Abernethy (1) reported the synthesis of hs proteins was induced by a hs within the initial 2 h of imbibition of isolated wheat embryos. We are interested in tolerance to environmental stress during early germination, particularly with reference to seedling vigor. The objective of the present study was to determine if the induction of hs protein synthesis conferred thermotolerance during the initial hours of wheat seed imbibition.

We report here that during early imbibition the wheat seed has substantial high temperature tolerance relative to later times of imbibition. Prior to periods of 9 to 12 h of imbibition, mild heat pretreatments are unable to elicit thermotolerance despite inducing a broad range of hs protein synthesis. Follow-
ing longer periods of imbibition, the initial high temperature tolerance declines and thermotolerance can be acquired. During this transition period, the hs induced synthesis of four developmentally regulated proteins gradually ceases. Their role, if any, in the acquisition of thermotolerance is unknown at present.

MATERIALS AND METHODS

Plant Material

Wheat (Triticum aestivum L. cv Lancer and cv Guard) seed was obtained from the University of Wyoming wheat program. Intact, uniform caryopses (referred to as seed) were hand selected for experiments. Embryos were isolated by the method of Johnston and Stern (10) when required. Intact, undamaged embryos were selected under 20x stereoscopic magnification. Imbibition solutions for intact seed contained 0.2% Thiram (tetramethylthiuram disulfide). IM for isolated embryos contained 1% sucrose and 1 μg ml⁻¹ chloramphenicol. Heat shock temperatures (51° or 52°C) were determined by preliminary experiments for each seedlot. A temperature lethal to >50% of the seed or embryos was desired.

Seed Acquisition of Thermotolerance

To assess the occurrence of thermotolerance during early imbibition, four replications of 20 seed were imbibed at 25°C for various lengths of time up to 16 h. The cultivar Lancer was used. Three hs temperature regimes were employed at the end of this imbibition time: (a) 38°C controls were given a 2 h 38°C treatment and transferred back to 25°C for 123 h, (b) 51°C controls were given a 2 h 51°C treatment and transferred back to 25°C for 123 h, and (c) 38°C → 51°C treatments were given a 2 h 38°C hs protein induction treatment followed by 1 h at 25°C, subsequently followed by the 2 h 51°C treatment, then maintained at 25°C for 120 h. Immediately following the temperature treatments, seed were carefully placed between two layers of germination toweling, rolled and placed upright into beakers of water for the 120 or 123 h growth period at 25°C. At the end of the growth period, lengths of coleoptile, plumule, radicle, and individual adventitious roots were measured for individual germinated seeds. Means, standard deviation and error, and confidence intervals (CI) were determined for each experiment.

Isolated Embryo Acquisition of Thermotolerance

Isolated embryos were imbibed in IM following the same protocol as for whole seed with the following modification. Following the 52°C hs, embryos were maintained at 25°C for 1 h then transferred to a TZ solution (0.02% w/v) for 2 h. The TZ treatment was conducted in darkness. Embryos were then washed with deionized water and dried under vacuum overnight before extraction of the TZ reduction product, formazan, by homogenization and 2 h extraction of the tissue in methylcellulose. Absorbance of the supernatant was determined at 480 nm. Preliminary experiments indicated this procedure minimized sample to sample variability.

Germination Temperature Tolerance

Four replications of 20 intact seed each were imbibed for durations ranging from 0 to 21 hr. Seed were imbibed on two layers of Whatman 2 paper in paraffin sealed Petri dishes. Dishes were maintained at 25°C in a darkened seed germination chamber. Following the initial imbibition period, dishes were transferred to a dark growth chamber for a 2 h hs at 48°, 50°, 52°, 54°, 56°, or 58°C, then returned to 25°C. Germinated seed were counted at 24 h intervals for 8 d.

Heat Shock Protein Synthesis

Labeling experiments were conducted with isolated embryos and whole seed. After various periods of imbibition in IM at 25°C, 6 to 8 embryos were transferred to a 15 μl droplet of IM containing [35S]methionine (7 μCi per embryo; >1000 Ci mmol⁻¹) then maintained at 25°C or 40°C for an additional 1.5 h in a water saturated atmosphere. Embryos were then rinsed twice in IM plus 1 mm methionine and homogenized in extraction buffer (62.5 mm Tris, 10 mm DTT, 2% SDS, 10% glycerol, pH 7.0). Intact-seed embryos were labeled by immersing the embryo end into a 7.5 μl droplet of [35S]methionine in wells of a microtiter plate, placed into a vacuum chamber for 2 min and then maintained at the desired temperature for 2 h. Embryos were then blotted, rinsed and rapidly dissected free of endosperm tissue, frozen in an isopropanol/dry ice bath, and finally homogenized. A clarified supernatant was obtained by centrifugation in a microfuge. Incorporation of radioactivity into TCA-insoluble material was determined by the method of Mans and Novelli (15). Extracted soluble proteins were analyzed electrophoretically in one dimensional SDS-PAGE by the method of Laemmli (12) as modified by Petersen and Mitchell (24). Samples of equal radioactivity (cpm) were brought to a volume of 20 μl with sample buffer (12) and loaded onto each lane of a gel. A current of 13 mA was applied for approximately 16 h. Following electrophoresis, gels were fixed, stained with Coomassie blue, and dried. Autoradiography was conducted by exposing the dried gels to Kodak SB-5 X-Ray film for 7 to 21 d, depending on the amount of radioactivity present. Two dimensional electrophoresis was done as described by O’Farrell (20) with the first dimension slightly modified according to Buzin and Petersen (4). Instead of using pH 5 to 8 ampholytes, pH 5 to 7 (Bio-Rad) ampholytes were mixed with pH 3 to 10 to provide a gradient over the approximate pH range of 4 to 7. When running isoelectric focusing gels, 300 V were applied to 10 tubes (2.3 × 85 mm) for 21 h. The lower chamber of the isoelectric focusing apparatus was cooled to 19°C. The low voltage and slight cooling resulted in a very linear pH gradient with little smearing in the basic portion of the gels.

RESULTS

Acquisition of Thermotolerance

Thermotolerance acquisition during early germination of intact wheat seed was evaluated by both viability and seedling growth responses after a 5 d growth period following hs treatments. Thermotolerance in these experiments was de-
fined as a significant increase in germination for the protected treatment over the 51°C heat shock treatment. Provision of a moderate, pretreatment temperature stress of 38°C, 40°C, or 42°C (Table I, treatment a) alone had minimal effect on germination throughout the initial 16 h of imbibition. Imposition of a 51°C hs for 2 h after the tested intervals of imbibition from 3 to 16 h reduced seed germination (viability) attained after 120 h to near the desired 50% level (Table I, treatment b). A definite trend toward decreased viability with greater times of imbibition prior to the hs was evident. High variation in seed survival following the 51°C hs is attributed to the severity of the stress. Slight variations in initial seed moisture content and individual seed vigor, and treatment application appeared to play a profound role in survival at this near lethal temperature. A protective effect of a 2 h pretreatment at 38°C to 42°C prior to the 2 h-51°C hs did not occur until 9 to 12 h of imbibition had elapsed. At 9 h and subsequently, thermotolerance was acquired after provision of the 38°C and 40°C pretreatments. With the 42°C pretreatment, thermotolerance acquisition was delayed until 12 h.

A similar requirement for the acquisition of thermotolerance was obtained when seedling growth parameters of surviving, germinated seedlings were measured at 5 d following the same temperature treatments (Table II). The protective effect of 38°C, 40°C, and 42°C treatments prior to a 51°C hs was reflected in significant increase in measured seedling growth parameters only after 9 to 12 h of imbibition. Prior to this time there was no difference between protected and unprotected treatments, i.e. thermotolerance was not acquired. If the individual seedling organs measured responded differently to the stress, it was not evident due to the variability in growth responses obtained.

To further evaluate the inability to obtain a thermotolerant response prior to 9 to 12 h of imbibition, additional experiments were conducted. These used the same treatment protocol described above with a 38°C pretreatment and with 52°C and 53°C hs after 6 h of imbibition. Preliminary experiments had indicated these temperatures were lethal to greater than 50% of the seed when applied during early imbibition. Thermotolerance was again not obtained at 6 h of imbibition, as assessed by germination or seedling growth response (Table III), at these higher temperatures.

The use of embryos isolated in bulk from seed tissue provides a system in which protein synthesis is readily monitored by labeled amino acid uptake. To evaluate the acquisition of thermotolerance of isolated embryos the accumulation of formazan, the reduced form of TZ, was measured following moderate and high stress heat treatments. Embryo germination and growth response was found difficult to evaluate, and the use of TZ reduction to assess seed viability has been widely used. Although sample variation was high, when 95% CI values for the means were used to compare treatments, it was evident that isolated embryos were also unable to acquire thermotolerance during the initial 6 h of imbibition (Table IV). After 6 h of imbibition a 38°C pretreatment provided increased tolerance to a 52°C hs. The earlier timing of thermotolerance acquisition by isolated embryos when compared to seed is attributed to an increased rate of water uptake by the embryos. This would result in a greater rate of increase for all components of germination metabolism (3). Results of these experiments substantiated the intact seed results, and justify the use of isolated embryos to study the relationship between thermotolerance acquisition and the hs response during the germination process. For convenience in discussion, the imbibition time required for the germinating seed to acquire thermotolerance has been termed the 'thermotolerance transition.'

### Early Germination High Temperature Tolerance

Both seed and isolated embryos exhibited greater tolerance to the high temperature shock during the initial hours of

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**Table I. Acquisition of Thermotolerance during Early Imbibition of Wheat Seed Assessed by Seed Viability**

Seed were imbibed at 25°C for intervals ranging from 3 to 16 h prior to receiving treatments of (a) 2 h heat pretreatment only, (b) 2 h hs at 51°C with no pretreatment, or (c) a 2 h pretreatment, 1 h at 25°C and by 2 h at 51°C. Seed viability was determined 120 h later by germination assessed by radicle protrusion of 3 mm through the seed coat. Thermotolerance was defined as an increase in germination percentage of treatment (c) over that of treatment (b).

<table>
<thead>
<tr>
<th>Temperature Treatment</th>
<th>Hours of Imbibition Prior to Treatment</th>
<th>% Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>(a) 38°C only</td>
<td>100 ± 0.0*</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>(b) 51°C only</td>
<td>69 ± 8</td>
<td>43 ± 5</td>
</tr>
<tr>
<td>(c) 38°C–51°C</td>
<td>55 ± 5</td>
<td>50 ± 10</td>
</tr>
<tr>
<td>(a) 40°C only</td>
<td>100 ± 0</td>
<td>98 ± 1</td>
</tr>
<tr>
<td>(b) 51°C only</td>
<td>70 ± 3</td>
<td>41 ± 7</td>
</tr>
<tr>
<td>(c) 40°C–51°C</td>
<td>59 ± 5</td>
<td>39 ± 7</td>
</tr>
<tr>
<td>(a) 42°C only</td>
<td>98 ± 2</td>
<td>98 ± 1</td>
</tr>
<tr>
<td>(b) 51°C only</td>
<td>56 ± 5</td>
<td>50 ± 5</td>
</tr>
<tr>
<td>(c) 42°C–51°C</td>
<td>48 ± 7</td>
<td>34 ± 6</td>
</tr>
</tbody>
</table>

* Values represent the mean and se of four replicate samples of 20 seeds each. Underlined values represent significantly increased survival of treatment (c) compared with treatment (b).
Table II. Acquisition of Thermotolerance during Early Imbibition of Wheat Seed Assessed by Seedling Growth Measurements

Seed were imbibed at 25°C for intervals from 3 to 16 h prior to receiving treatments (a) a 2 h heat pretreatment at 38, 40, or 42°C; (b) a 2 h heat at 51°C, or (c) 2 h at 38, 40, or 42°C pretreatment followed 1 h later by 2 h at 51°C. Seedling growth parameters that included total adventitious root, coleoptile, radicle, and shoot lengths were measured 120 h later. Thermotolerance is defined as a significant increase (based on the 95% CI) in growth of treatment (c) over that of treatment (b).

<table>
<thead>
<tr>
<th>Imbibition h</th>
<th>Treatment</th>
<th>Total Seedling Root Length at hs Pretreatment (cm)</th>
<th>Coleoptile Length at hs Pretreatment (cm)</th>
<th>Radicle Length at hs Pretreatment (cm)</th>
<th>Shoot Length at hs Pretreatment (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>(a) 297* (7.6)* 363 (14.6) 339 (16.2) 38 (0.5) 57 (1.3) 56 (1.3) 100 (4.4) 116 (7.9) 100 (8.5) 65 (1.8) 21 (2.1) 17 (2.2)</td>
<td>100 (4.4) 116 (7.9) 100 (8.5) 65 (1.8) 21 (2.1) 17 (2.2)</td>
<td>40 (3.8) 73 (7.0) 61 (7.4) 2 (1.2) 5 (1.2) 5 (2.2)</td>
<td>43 (3.7) 69 (8.1) 68 (9.1) 2 (1.2) 4 (1.3) 5 (1.8)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>(a) 237 (6.9) 410 (13.1) 385 (14.5) 31 (0.8) 55 (1.1) 49 (1.0) 87 (3.3) 133 (7.5) 124 (7.3) 50 (2.1) 26 (2.6) 30 (2.9)</td>
<td>87 (3.3) 133 (7.5) 124 (7.3) 50 (2.1) 26 (2.6) 30 (2.9)</td>
<td>42 (5.3) 50 (9.8) 45 (8.4) 6 (2.8) 6 (2.3) 7 (2.4)</td>
<td>43 (4.7) 48 (10.0) 63 (12.7) 11 (3.1) 3 (1.6) 11 (3.7)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>(a) 294 (9.0) 407 (16.3) 383 (17.0) 34 (0.8) 49 (0.9) 49 (1.0) 96 (4.7) 130 (8.3) 113 (8.8) 70 (2.5) 36 (3.6) 31 (3.3)</td>
<td>96 (4.7) 130 (8.3) 113 (8.8) 70 (2.5) 36 (3.6) 31 (3.3)</td>
<td>32 (6.0) 42 (22.2) 48 (10.9) 13 (3.3) 12 (7.5) 6 (2.6)</td>
<td>35 (5.5) 27 (7.8) 58 (20.4) 28 (3.3) 8 (3.4) 22 (7.6)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>(a) 279 (6.3) 425 (13.7) 397 (15.1) 34 (0.7) 45 (0.9) 47 (1.0) 87 (3.6) 140 (6.5) 116 (8.5) 70 (2.4) 35 (3.0) 32 (2.7)</td>
<td>87 (3.6) 140 (6.5) 116 (8.5) 70 (2.4) 35 (3.0) 32 (2.7)</td>
<td>34 (4.9) 21 (6.1) 37 (8.9) 10 (2.9) 2 (0.9) 5 (2.1)</td>
<td>62 (4.7) 32 (8.5) 26 (8.5) 38 (2.6) 11 (2.2) 7 (1.9)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>(a) 320 (16.6) 430 (17.0) 439 (15.6) 43 (1.0) 38 (0.9) 43 (0.5) 109 (6.1) 139 (8.1) 145 (8.3) 27 (5.1) 36 (3.3) 38 (3.0)</td>
<td>109 (6.1) 139 (8.1) 145 (8.3) 27 (5.1) 36 (3.3) 38 (3.0)</td>
<td>33 (7.1) 51 (37.7) 25 (8.8) 6 (2.0) 4 (1.4) 2 (1.1)</td>
<td>73 (7.3) 41 (6.8) 28 (6.3) 12 (2.5) 16 (2.3) 8 (1.7)</td>
<td></td>
</tr>
</tbody>
</table>

*Values represent the mean of germinated seedlings only from four replicates of 20 seeds each. Underlined values represent significantly increased growth of treatment (c) when compared with treatment (b).

Table III. Inability of Wheat Seed to Acquire Thermotolerance to 52 or 53°C hs at 6 h of Imbibition Evaluated by Both Seedling Growth and Viability

Seedling growth and germination of 38°C controls, 52 or 53°C hs at 38°C and 38°C heat pretreated for 2 h followed by 2 h at 52 or 53°C hs treatments was measured 5 d later. All temperature treatments were imposed after 6 h imbibition at 25°C. Values represent the mean of four replicates of 20 seeds each.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Length</th>
<th>Radicle</th>
<th>Coleoptile</th>
<th>Shoot</th>
<th>Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>cm</td>
<td>%</td>
<td>cm</td>
<td>cm</td>
<td>cm</td>
<td>%</td>
</tr>
<tr>
<td>38°C</td>
<td>390 (16.2)* 141 (6.3) 54 (1.5) 32 (4.4) 86</td>
<td>86</td>
<td>54 (1.5) 32 (4.4) 86</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>52°C hs</td>
<td>200 (40.8)* 68 (11.0) 45 (5.0) 5 (2.8) 16</td>
<td>16</td>
<td>68 (11.0) 45 (5.0) 5 (2.8) 16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>38°C→52°C</td>
<td>168 (44.9) 67 (14.1) 41 (5.7) 4 (2.6) 16</td>
<td>16</td>
<td>67 (14.1) 41 (5.7) 4 (2.6) 16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>38°C</td>
<td>324 (17.5) 108 (6.1) 54 (1.7) 31 (4.4) 90</td>
<td>90</td>
<td>108 (6.1) 54 (1.7) 31 (4.4) 90</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>53°C hs</td>
<td>257 (30.0) 85 (9.6) 48 (3.6) 8 (3.0) 21</td>
<td>21</td>
<td>85 (9.6) 48 (3.6) 8 (3.0) 21</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>38°C→53°C</td>
<td>274 (44.1) 93 (11.0) 54 (2.9) 14 (7.0) 10</td>
<td>10</td>
<td>93 (11.0) 54 (2.9) 14 (7.0) 10</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

*Values in parentheses represent a 95% CI about the mean.

RESULTS

The occurrence of hs protein synthesis during the time of imbibition prior to the thermotolerance transition was shown results were obtained (data not shown). This tolerance to high temperature during early imbibition has not previously been reported. Due to the relatively greater tolerance to high temperature during early imbibition, the thermotolerance transition apparently involves the loss of an existing thermotolerance coincident with acquiring the ability to become thermotolerant following heat pretreatment.

The primary manifestation of the tolerance appears to be the ability of seed to survive a hs, and secondarily the response can be attributed to a transient delay in germination processes. The ability to survive decreases with time of imbibition and severity of the hs. Data in Figure 1 show that the rate (slope of the cumulative germination percentage line) of germination after 2 hs up to 52°C at 0, 3, 6, and 9 h of imbibition was essentially equal until 72 h of imbibition. Not until the 21 h hs was the germination rate noticeably reduced. At hs temperatures above 52°C both time of initiation and rate of germination were reduced as the time of imbibition prior to receipt of the hs increased. This indicates that during at least the initial 6 h of imbibition the rate of events leading to germination was not significantly altered by hs up to 52°C. As the hs temperature increases above this temperature the probability of injury leading to reduced rate of essential processes is increased. The identity of these essential processes is unknown but of interest to us.

hs Proteins

The occurrence of hs protein synthesis during the time of imbibition prior to the thermotolerance transition was shown...
**Table IV. Isolated Wheat Embryo Acquisition of Thermotolerance, Assessed by T2 Viability, Can Occur at 8 h of Imbibition**

Isolated wheat embryos were imbibed at 25°C for intervals ranging from 2 to 10 h prior to receipt of one of three heat treatments: (a) 2 h at 38°C, (b) 2 h at 52°C, (c) 2 h at 38°C followed 1 h later by 2 h at 52°C. At the end of the heat treatment samples were transferred to 25°C for 1 h prior to incubation with T2 for 2 h. Absorbance of the formazan reduction product was determined after extraction. Values represent the mean and SE of 6 samples of 10 embryos each.

<table>
<thead>
<tr>
<th>Heat Treatment</th>
<th>Hours of Imbibition at 25°C Prior to hs</th>
<th>A490</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>(a) 38°C</td>
<td>0.045 (± 0.015)</td>
<td>0.052 (± 0.014)</td>
</tr>
<tr>
<td>(b) 52°C</td>
<td>0.108 (± 0.008)</td>
<td>0.026 (± 0.015)</td>
</tr>
<tr>
<td>(c) 38°C—52°C</td>
<td>0.027 (± 0.017)</td>
<td>0.045 (± 0.033)</td>
</tr>
</tbody>
</table>

**Figure 1.** Decreasing tolerance to high temperature during imbibition of intact wheat seed as shown by cumulative germination over 192 h. Seed were given 2 h hs that ranged from 48° to 58°C at 2°C intervals. The hs were administered at the initiation of imbibition (0 h), and after 3, 6, 9, and 21 h of imbibition at 25°C. Bars represent 1 SD about the mean.

In earlier studies (1). To evaluate the spectrum of hs proteins synthesized by isolated embryos before and after the thermotolerance transition in greater detail, two-dimensional gel separations of 25°C protein synthesis and 38°C hs protein synthesis at 1.5 and 12 h of imbibition were compared (Fig. 2). There was a strong hs response as early as 1.5 h of imbibition in isolated wheat embryos, although we have shown that additional thermotolerance cannot be acquired at this time. When the hs protein spectrum of 1.5 and 12 h imbibed embryos was compared, a developmentally regulated hs response was observed. The hs proteins of 58 to 60, 46, 40, and 14 kD were detected at 1.5 h of imbibition following hs, but were absent or greatly reduced at 12 h of imbibition. Additionally, when comparing protein synthesis of 1.5 and 12 h imbibed embryos at 25°C, there appeared to be a consistently higher level of synthesis of the hs proteins of 68 to 70 family, a group of hs proteins of 94 to 96 kD, and possibly others at 1.5 h.

**DISCUSSION**

Developmental control of the hs response during specific embryogenic stages of Xenopus, Drosophila, and mammalian cells has been recognized for some time (9, 19). In these organisms, hs of 15 to 60 min duration produced high lethality during specific developmental stages. There was no transcriptional induction of hs protein mRNA, and concomitant hs protein synthesis was not observed. The experiments reported here demonstrate a very different response.

Pronounced hs protein synthesis representing a broad spectrum of hs proteins occurs during early imbibition of the wheat seed and yet thermotolerance could not be acquired until several hours of imbibition had elapsed. However, this time interval correlated with a period in which the germinating seed already exhibited a greater tolerance to high temperatures than at later times of imbibition. Due to the unique nature of the observed response in relation to other known hs responses, a broad range of temperatures were used for both initial moderate heat stress pretreatments and for the near lethal temperature treatments. It is known that the protective response associated with hs protein synthesis induction in Drosophila is highly dependent on the degree of temperature stress (18), and it was considered important to evaluate this possibility in the germinating seed. Higher pretreatment temperatures during early imbibition of wheat seed tended to result in an additive response to that of the second hs. Lethality was increased by higher pretreatment temperatures. No pretreatment/hs temperature combination studied resulted in the acquisition of thermotolerance by imbibing wheat seed or isolated embryos prior to 12 or 8 h of imbibition, respectively.

Acquisition of thermotolerance provided by a 38°C pretreatment administered after the thermotolerance transition was manifested in both increased survival and increased elon-
growth of the seedlings compared to that without a 38°C pretreatment (Tables I and II). Maintenance of viability was the more significant response. However, when a 2 h hs up to 52°C was received during the initial 6 h of imbibition, no delay in germination rate was observed (Fig. 1), but after 6 h of imbibition or at higher temperatures the germination rate was delayed. This finding suggests that whatever protective function the pretreatment provides may either prevent the cause of this delay in germination or allow a partial recovery in seedling growth processes following the hs.

The tolerance to high temperature during the initial hours of imbibition, relative to later stages, may have causal significance with respect to the inability to obtain further protection to high temperature even in the presence of hs protein synthesis. The basis for this tolerance and its diminution with increasing time of imbibition is of great interest, but the number of possible explanations is many. Developmental loss of expression of several hs proteins (Fig. 2), which correlates to some degree with the reduction in high temperature tolerance and the ability to acquire thermotolerance, is intriguing. However, the relation of these proteins to thermotolerance is difficult to study in this higher plant system in which site-directed mutagenesis, transformation, and regeneration are not currently successful.

Several additional testable hypotheses are under consideration. Constitutive hs protein synthesis, via translation of a conserved hs protein mRNA fraction in the dry seed, may be a normal developmental process during early germination. Some of these proteins may be responsible for the observed high temperature tolerance. Evidence for this can be seen in the 25°C, 1.5 h imbibition polypeptide profile of Figure 2, in which both hs protein 68 to 70 and hs protein 94 to 96 groups suggest increased synthesis relative to that at 12 h imbibition. Furthermore, Northern analyses done in our laboratory show the presence of hs protein 70 mRNA in dry wheat seed (unpublished data). Dr. E. Vierling (personal communication) has also obtained evidence for low mol wt hs protein mRNAs in dry pea seed. The occurrence of conserved mRNAs in the
mature, dry wheat embryo is well known although the protein products and their functions remain obscure. Ribosomal proteins and a methionine-rich polypeptide, the Em protein, are among the few proteins identified (26). Embryogenesis-specific mRNAs such as the Em polypeptide are known to decrease rapidly after the onset of germination (29).

An additional hypothetical basis for early imbibition high temperature tolerance is supported by recent reports of differential sensitivity of cell cycle phases to heat stress in mammalian cell cultures. The DNA synthesis (S) phase of the cell cycle is more sensitive to hs than G1 or G2 phases. Sherwood et al. (25) suggested that the effect of cycloheximide in hs Chinese hamster ovary cell cultures may be to alter the cell cycle phase distribution or progression through S phase through its known effects on inhibition of DNA synthesis. In asynchronous tomato cell cultures, hs resulted in cell cycle arrest and reversal during recovery (24). Initiation of DNA replication in the imbibing wheat embryo has been reported to occur at 4, 12, and 15 h in different reports (reviewed in Ref. 21), but it is clear that DNA replicative synthesis is not initiated until some time of imbibition has elapsed. Evidence has been presented that synthesis of wheat embryo DNA polymerase was not initiated until 6 h of imbibition, and necessarily preceded replication (21). The possibility that the thermotolerance transition and initiation of DNA replication coincide seems worthy of further consideration.

Another possible basis for the high temperature tolerance prior to the thermotolerance transition could be the presence of hs proteins themselves in the mature dry embryo. This hypothesis is readily testable by the use of antibodies specific to selected hs proteins. The involved hs proteins could be synthesized as a normal part of seed development and retained during seed desiccation and early germination, during which time they undergo degradation. Alternately, they may be synthesized only in response to high temperature stress during seed maturation. In this instance seed that matured in an environment in which heat stress was absent would not be expected to contain the hs proteins. It is likely that, some time during the maturation of wheat seed, temperatures of 38° to 40°C would be experienced in most areas of commercial seed production. The seed lots used in this study have a high probability of experiencing hs protein inducing temperatures during development, although data to verify this is unavailable.

The above hypotheses relative to the basis for high temperature tolerance during early imbibition of wheat seed are admittedly speculative. Other hypotheses can be advanced, however, active pursuit of those discussed are underway.

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