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Heat Shock Proteins in Two Lines of *Zea mays* L. That Differ in Drought and Heat Resistance[1]

Zoran Ristic*[2], David J. Gifford, and David D. Cass
Department of Botany, University of Alberta, Edmonton, Alberta, Canada, T6G 2E9

ABSTRACT

Synthesis of heat shock proteins (HSPs) in the leaves of a drought- and heat-resistant (line ZPBL 1304), and a drought- and heat-sensitive (line ZPL 389) line of maize (*Zea mays* L.) was studied under two environmental stress treatments: (a) soil drying and high temperature and (b) high temperature. In the first treatment 13-day-old plants were exposed to 7-day soil drying followed by high temperature stress (45°C), and in the second treatment 20-day-old plants were exposed to high temperature stress (45°C). Second leaves were labeled with [^{35}S]methionine. During the labeling period line ZPBL 1304 showed no signs of leaf dehydration under soil drying and high temperature stress conditions. In contrast, line ZPL 389 was dehydrated 23%, as determined by relative water content. Incorporation of [^{35}S]methionine into protein was greater in the resistant than in the sensitive line in both treatments. The pattern of synthesis of HSPs in the two lines was similar in treatments 1 and 2. Both lines synthesized a high molecular mass set and a low molecular mass set of HSPs. Proteins from both sets from both lines of maize appeared similar to each other, with respect to the molecular mass. Heated plants of the drought- and heat-resistant line ZPBL 1304 synthesized a band of HSP(s) of approximately 45 kilodaltons which was not found in heated plants of the drought and heat sensitive line ZPL 389. This is the first report on qualitative intraspecific difference in the synthesis of HSPs in maize.

Heat shock induces the synthesis of HSPs[3] and, at the same time, suppresses the synthesis of the normal complement of cellular proteins (19). The synthesis of HSPs has been observed in a variety of plant species, and the general phenotype of the heat shock response is highly conserved in all organisms (14). Although the function of HSPs is not clear, it is generally believed that they play a role in thermal resistance (14, 19).

It is reasonable to expect that inter- and intraspecific differences in the pattern of synthesis of HSPs between plants which differ in heat resistance exist. However, this has not been found in many species to date. The thermal-resistant *Triticum aestivum* L. cv Mustang synthesized unique HSPs that were absent in the thermal-sensitive *T. aestivum* cv Sturdy (16). In contrast, no qualitative differences in HSPs were observed between heat resistant *Gossypium barbadense* L. line 7456 and heat sensitive *Gossypium hirsutum* L. line Paymaster 404 (8). Similarly, no differences in the pattern of synthesis of HSPs were found between 24-h-old seedlings of high temperature-resistant and high temperature-susceptible lines of *Sorghum bicolor* Moench (23). In addition, a comparison of the profiles of HSPs synthesized in the roots between heat-resistant and heat-susceptible cultivars of maize (*Zea mays* L.) did not reveal any qualitative intraspecific differences (6).

In most of the studies on heat shock-induced HSPs in plants, plant cells, tissues, organs, and in particular whole plants, have been exposed to only one environmental stress factor: high temperature. This experimental approach, however, may not reflect the conditions that plants may experience in the field. Under field conditions plants are often simultaneously exposed to soil drying, and high temperature stress. These two stress factors could create water deficit in plant tissues, which, in turn, may affect the synthesis of HSPs. The synthesis of HSPs under both soil drying and high temperature stress conditions has been observed in *G. hirsutum* L. (4) and *Glycine max* (L.) Merr. (15).

One of the differences in drought resistance between some drought-resistant and drought-sensitive plants is the difference in dehydration avoidance. Our preliminary studies of leaf dehydration in the drought-resistant line of maize ZPBL 1304 and drought-sensitive line ZPL 389 (25) indicated that the resistant line had a much greater ability to avoid dehydration under soil drying and high temperature stress conditions than the sensitive line (Z. Ristic, D.D. Cass, unpublished results).

We speculate that differences in the pattern of synthesis of HSPs between plants which differ in dehydration avoidance and heat resistance could exist in the leaves. When exposed to high temperature, leaves from the plants with greater dehydration avoidance will either not be dehydrated or will be dehydrated less than leaves from plants with lower dehydration avoidance. Hence, the RWC in the leaf tissues of such plants may have an impact on the synthesis of HSPs. The objectives of this study were to examine the synthesis of HSPs in the leaves of two lines of maize which differ in dehydration avoidance and heat resistance under two environmental stress treatments: (a) soil drying and high temperature and (b) high temperature alone.

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[2] Present address: Department of Horticulture, Horticulture Building, Purdue University, West Lafayette, IN 47907.

[3] Abbreviations: HSPs, heat shock proteins; RWC, relative water content; DHR, drought and heat resistant; DHS, drought and heat sensitive.

MATERIALS AND METHODS

Plant Material

Two lines of maize (*Zea mays* L.) were chosen: ZPBL 1304 and ZPL 389. Lines ZPBL 1304 and ZPL 389, relative to each other, have been classified as drought resistant and drought sensitive, respectively (25). Drought classification was based on the results from several tests of visual assessment of plant response to drought in the field, and seed germinability in the presence of high osmotica (25).

Assessment of Dehydration Avoidance and Heat Resistance

Two experiments were run: experiment A (six replicates) and experiment B (five replicates). Growth conditions were similar to those described by Ristic and Cass (27). The experimental set up was the same for both experiments unless otherwise stated. For each line 11 kernels were sown in each of two pots (pot diameter at the top and the bottom 20.5 cm and 14 cm, respectively; pot height 20 cm) containing a mixture of soil:peat:sand (3:1:1, v/v). Plants were grown under controlled environmental conditions (12 h photoperiod; 280 μmol m^{-2} s^{-1} PPFD; 24°C/18°C day/night temperature; 70% RH day and night) and were watered daily up to the second leaf stage (for 13 d). Plants were then divided into control (one pot) and experimental groups (one pot). The experimental group was not watered for 7 d. On the seventh day of imposed soil drying plants from the experimental groups were exposed to 45°C heat shock for 6 h (experiment A), and 24 h (experiment B). Temperature was gradually increased from 24°C to 45° over 1 h. Exposure time for heat shock treatment was measured from the moment when the temperature reached 45°C. After heating, plants were rewatered and allowed to recover for 6 d. Samples for RWC and plasmalemma damage were obtained from the second leaf blades collected from two randomly selected plants from each pot. Relative water content was determined 2.5 h after the beginning of heat shock (experiment A) immediately after the heat shock, and on the sixth day of recovery (experiments A and B). Damage to the plasmalemma was determined at the end of heating period, and on the sixth day of recovery (experiments A and B). All the replicates from each experiment were used to determine RWC and plasmalemma damage. Relative water content was determined according to Henson *et al.* (10), and damage to the plasmalemma was assessed using the method of Pearce (24).

Synthesis of HSPs in Plants Exposed to Soil Drying and High Temperature Stress (Treatment 1)

Plants from experiment A were used to study the synthesis of HSPs under soil drying and high temperature stress conditions. The temperature of 45°C was chosen because the work of Cooper *et al.* (5) showed that for intact maize plants this was the optimal temperature for the synthesis of HSPs. Two hours after the beginning of heat shock, one plant randomly selected from each of the experimental and control groups was radioactively labeled for 1 h to determine the pattern of synthesis of HSPs. Plants in three replicates were radioactively labeled, and labeling was performed simultaneously in the control and the experimental plants.

Synthesis of HSPs in Plants Exposed to High Temperature Stress (Treatment 2)

For each line 11 kernels were sown in each of two pots. Experimental set up and growth conditions were identical to those in experiment A with the exception that plants were watered for 20 d. Plants were then divided into control (one pot) and experimental groups (one pot), and plants from the experimental group were exposed to 45°C heat shock. Heat shock treatment and labeling were identical to those in experiment A. During the heat shock treatment plants were kept well watered, and no visible signs of wilting were noticed.

In Vivo Labeling and Extraction of Proteins

In vivo labeling and extraction of proteins was performed as described by Cooper *et al.* (5). The second leaf blades of intact plants were labeled by first lightly abrading a small area of adaxial leaf surface with emery paper, then applying 10 μL of [^{35}S]methionine (3.89 GBq/10 μL; specific activity 38.9 TBq/mmol; Amersham, Canada) to this surface. The labeled region was covered with cellophane to prevent evaporation of label and drying of the leaf tissue. Following labeling, the labeled portion of the leaf was cut and homogenized in 500 μL SDS Laemmli buffer (18). The homogenate was heated in a water bath at 95°C for 5 min, and then centrifuged at 10,000*g* for 15 min. The supernatant was analyzed for incorporation of [^{35}S]methionine into proteins by TCA precipitation (20).

Gel Electrophoresis of Proteins and Autoradiography

Radioactively labeled proteins from two replicates from treatment 1, and one replicate from treatment 2 were analyzed separately for each replicate by SDS-PAGE (18). Gels were loaded with equal amounts of TCA precipitable counts. Along side the extracted proteins the following ^{14}C methylated standards were run: myosin (200 kD), phosphorylase-B (92.5 kD), BSA (69 kD), ovalbumin (46 kD), carbonic anhydrase (30 kD), and lysozyme (14.3 kD) (Amersham, Canada). Gels were fixed in 200 mL of 30% (v/v) methanol and 10% (v/v) acetic acid, enhanced in 100 mL/gel autoradiography enhancer EN^{3}HANCE, and dried at 60°C. Autoradiography was carried out at −70°C using Kodak X-Omat AR film.

RESULTS AND DISCUSSION

Assessment of dehydration avoidance and heat resistance indicated a great difference between lines ZPBL 1304 and ZPL 389 (Z. Ristic, D.D. Cass, unpublished results). Line ZPBL 1304 showed much greater capability of avoiding dehydration and resisting heat shock than line ZPL 389, as indicated by RWC and damage to the plasmalemma. After heating for 6 h lines ZPBL 1304 and ZPL 389 had RWC 97% (±2% SE) and 56% (±8% SE), and after heating for 24 h 48% (±4% SE) and 11% (±2% SE), respectively. Damage to the

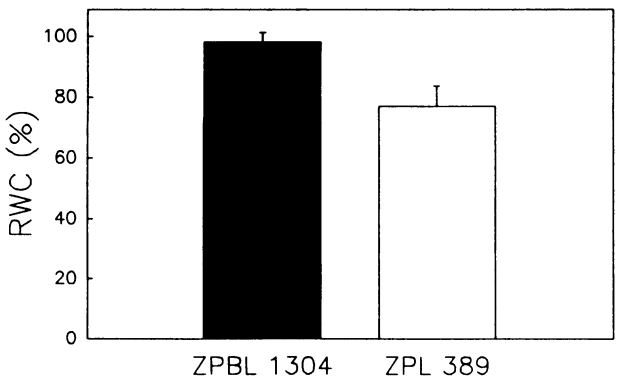

Figure 1. RWC in the second leaf blades of DHR (line ZPBL 1304) and DHS (line ZPL 389) lines of maize. Thirteen-day-old plants were exposed to 7 d of soil drying followed by 45°C heat stress. Relative water content was determined according to Henson *et al.* (10) 2.5 h after the beginning of heat stress. (n = 6; bars indicate SE).

plasmalemma after 6-h heat shock was negligible (1% ± 1% SE) in line ZPBL 1304, and 28% (±3% SE) in line ZPL 389. The 24-h heat shock injured plasmalemma in 18% (±2% SE) of line ZPBL 1304, and killed almost all the plants in line ZPL 389. Leaf dehydration, and damage to the plasmalemma were reversible in 6-h-heated plants of line ZPL 389, and 24-h heated plants of line ZPBL 1304; 6-d-recovered plants had RWC and damage to the plamalemma of 87% (±3% SE) and 2% (±1% SE), respectively, for ZPL 389, and 92% (±3% SE) and 3% (±2% SE), respectively, for ZPBL 1304.

The DHR line ZPBL 1304 and DHS line ZPL 389 differed in leaf dehydration during the labeling period of the synthesis of HSPs under soil drying and high temperature stress conditions (treatment 1). As indicated by the RWC 30 min after the labeling (Fig. 1), the DHR line showed no signs of dehydration. In contrast, the DHS line was dehydrated 23%.

Total protein synthesis during the labeling period was much greater in the DHR line ZPBL 1304 than in the DHS line ZPL 389 in both treatments. This was indicated by incorporation of [^{35}S]methionine into protein (Table I). The difference between the two lines was apparent in each replica, although the number of protein TCA precipitable counts varied between the replicates.

Differences in total protein synthesis between lines ZPBL 1304 and ZPL 389 under stress conditions were in agreement with previous work. In 12-h-old seedlings of *Sorghum bicolor* incorporation of [^{35}S]methionine into protein at 45°C and 47°C was much greater in the thermoresistant line IS18530 than in thermosensitive line IS4845 (11). Fedina *et al.* (7) also reported that protein synthesis in calli of *Glycine max* was much less affected in the more thermoresistant AR-2 line than in the less thermoresistant WT line.

Exposure to soil drying and high temperature stress induced the synthesis of HSPs in intact leaves from lines ZPBL 1304 and ZPL 389 (Fig. 2). The results from two replicates were qualitatively identical. Two sets of HSPs were apparent: a high molecular mass set (molecular mass >60 kD) and a low molecular mass set (molecular mass <30 kD). Heat shock proteins from both sets from both lines of maize appeared similar to each other, with respect to the molecular mass. Heat shock proteins of approximately 92 kD, 80 to 85 kD, 70 kD, 26 to 28 kD, and 18 to 20 kD were synthesized in both lines of maize.

The pattern of synthesis of HSPs in the two lines under heat shock treatment was similar to that under soil drying and high temperature stress (Fig. 3).

Although lines ZPBL 1304 and ZPL 389 had similar pattern of the synthesis of low and high molecular mass HSPs, some quantitative differences in the synthesis of HSPs between the two lines might exist. Visual comparison of the bands of HSPs of 18 to 20 kD, for example, from heated plants indicated that ZPL 389 synthesized greater amount of this (these) protein(s) than ZPBL 1304. This was observed in all replicates under both stress treatments. Further studies, however, are needed to address the question as to whether the two lines differ quantitatively in the synthesis of HSPs.

Some of the high and low molecular mass HSPs from the leaves from our maize lines were similar to those reported in other maize cultivars. Heat shock proteins of approximately 80 to 85 kD, 70 kD, 26 to 28 kD, and 18 to 20 kD were

Table I. *Radioactivity (dpm) of Incorporated [^{35}S]Methionine into Protein of Leaves in the DHR (ZPBL 1304) and the DHS (ZPL 389) Lines of Maize*

In replicas 1, 2, and 3, 13-d-old plants were exposed to 7 d of soil drying followed by 45°C heat stress. In replica 4, 20-d-old, well-watered plants were exposed to 45°C heat stress, and during the heat treatment were kept well watered. In all replicas, 2 h after the beginning of heat stress, the second leaf blades of one plant randomly selected from the control and one from the treatment were labeled with [^{35}S]methionine for 1 h. Incorporation of the label into protein was analyzed by TCA precipitation (20).

Replica No.	Line of Maize					
	ZPBL 1304			ZPL 389		
	Control	Treatment	Percent of control	Control	Treament	Percent of control
1	486,941	613,724	126	485,776	186,051	38
2	716,472	1,453,650	203	570,398	344,681	60
3	58,668	30,495	52	383,329	11,005	3
4	353,845	419,820	119	311,635	211,408	68

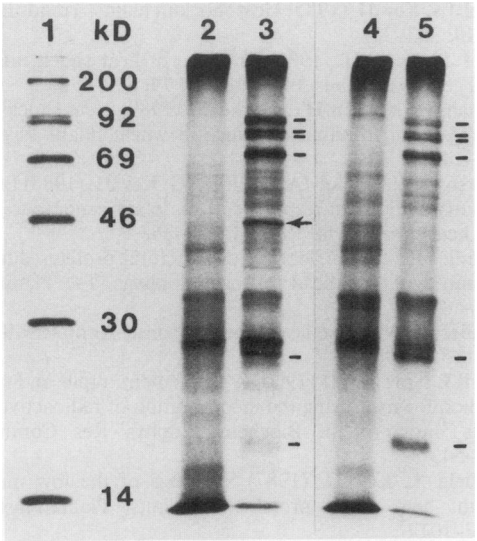

Figure 2. Pattern of synthesis of HSPs in DHR (line ZPBL 1304) and DHS (line ZPL 389) lines of maize. Thirteen-day-old plants were exposed to 7 d of soil drying followed by 45°C heat stress. Two hours after the beginning of heat stress, the second leaf blades of one plant randomly selected from the control and one from the treatment were labeled with [^{35}S]methionine for 1 h. Radioactively labeled proteins from two replicates were analyzed separately by SDS-PAGE (18). 1, standards; 2, ZPBL 1304, control; 3, ZPBL 1304, treatment; 4, ZPL 389, control; 5, ZPL 389, treatment. Bars indicate HSPs. Arrow indicates a unique 45-kD band of HSP(s).

found in the leaves of cultivar A632 × C1042 (5). HSPs of similar molecular mass were also synthesized in the roots of the same cultivar (5).

Significantly, a band of HSP(s) of approximately 45 kD appeared in the heated plants of DHR line ZPBL 1304 under both stress treatments (soil drying and high temperature stress, and high temperature stress) that was not observed in the heated plants of DHS line ZPL 389 (Figs. 2 and 3, indicated by arrows). However, a small amount of the protein(s) of approximately 45 kD was observed in the control plants of DHS line ZPL 389.

It can be argued that a qualitative difference in the pattern of synthesis of HSPs between lines ZPBL 1304 and ZPL 389 under soil drying and high temperature stress did not reflect the difference between the two genotypes, since the two lines differed in the degree of leaf dehydration. The synthesis of HSPs under heat shock conditions (treatment 2), however, clearly showed that the DHR line ZPBL 1304 and DHS line ZPL 389 differed qualitatively in the pattern of synthesis of HSPs.

The 45-kD band of HSP(s) we observed in the leaves of DHR line ZPBL 1304 appears to be uncommon in higher plants. Several studies of HSPs in higher plants have been carried out in maize (1, 3, 5, 12, 17, 21, 28, 30) and other species (2, 4, 11, 13, 15, 17, 21, 22, 29). In none of these was there any report on HSPs of 45 kD. HSPs which have molecular mass of approximately 43 kD have been reported in maize young seedling mesocotyls (cv Canada No. 1) (9), *Triticum aestivum* cv Mustang and cv Sturdy (42 kD) (16) and *G. max* cv Severnaya 3 (47 kD) (7). The only report of 45-kD HSP(s) of which we are aware was found in heat-shocked (38°C) protoplasts from suspension cultures, in embryos and regenerated plantlets of *Daucus carota* L. (26).

The appearance of a band of HSPs of 45 kD in the heated plants of DHR line ZPBL 1304, and a band of protein(s) of 45 kD in the control plants of DHS line ZPL 389 indicates that both lines have genes which encode protein(s) of this mass. However, the response of the two lines to the heat shock, with respect to the synthesis of this(these) protein(s), was different; line ZPBL 1304 synthesized the 45-kD band whereas line ZPL 389 did not. We do not know if 45-kD bands in the two lines are comprised of the same protein(s), and, therefore, further investigation is needed to give a better idea as to whether these bands could contain identical proteins. It is possible that the pattern of synthesis of HSPs in lines ZPBL 1304 and ZPL 389 has an impact on the drought and heat resistance in these two lines.

In summary, studies of HSPs in the DHR line of maize ZPBL 1304, and the DHS line ZPL 389 revealed qualitative differences in the pattern of synthesis of HSPs between the two lines. Total protein synthesis under stress conditions was greater in line ZPBL 1304 than in line ZPL 389. Both lines synthesized low molecular mass and high molecular mass HSPs which, with respect to the molecular mass, were similar. A unique 45-kD band of HSP(s) was found in the heated plants of DHR line ZPBL 1304. As far as we are aware, this band has not been previously described in the leaves of maize, and does not appear to be common in higher plants.

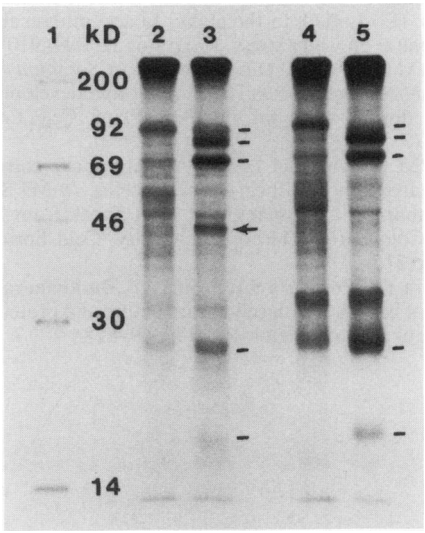

Figure 3. Pattern of synthesis of HSPs in DHR (line ZPBL 1304) and DHS (line ZPL 389) lines of maize. Twenty-day-old, well watered plants were exposed to 45°C heat stress, and during the heat treatment were kept well watered. Two hours after the beginning of heat stress, the second leaf blade of one plant randomly selected from the control and one from the treatment were labeled with [^{35}S] methionine for 1 h. Radioactively labeled proteins were analyzed by SDS-PAGE (18). 1, standards; 2, ZPBL 1304, control; 3, ZPBL 1304, treatment; 4, ZPL 389, control; 5, ZPL 389, treatment. Bars indicate HSPs. Arrow indicates a unique 45-kD band of HSP(s).

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