

## HEAT SHOCK OF CULTIVATED AND WILD WHEATS DURING EARLY SEEDLING STAGE: GROWTH, CELL VIABILITY AND HEAT SHOCK PROTEINS

M. YILDIZ<sup>1\*</sup> and S. TERZIOGLU<sup>2</sup>

<sup>1</sup>Department of Biology, ANS Campus, Faculty of Science and Arts, Afyon Kocatepe University,  
03100 Afyonkarahisar, Turkey

<sup>2</sup>Department of Biology, Beytepe Campus, Faculty of Science, Hacettepe University,  
06532 Ankara, Turkey

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Growth, cell viability and heat shock proteins (HSPs) in Bezostaya-1, Çukurova-86 and Diyarbakır-81 cultivated wheat cultivars and three *Aegilops* species were investigated. Etiolated seedlings were exposed to 23 °C, 32 °C, 35 °C, 37 °C and 38 °C for 24 h, and 35 °C (24 h) → 50 °C (1 h) and 37 °C (24 h) → 50 °C (1 h). At the end of recovery growth periods, the shoot lengths of the genotypes generally decreased significantly at 35, 37 and 38 °C. The acquired thermal tolerance (ATT) in intact seedlings was over 50% at 35 °C → 50 °C and 37 °C → 50 °C, but in cell viability test it ranged from 2.75% (*Ae. triuncialis*) to 32.87 (Bezostaya-1) at 35 °C, and from 2.82% (*Ae. triuncialis*) to 37.82 (Bezostaya-1) at 37 °C. *Ae. triuncialis* was most sensitive genotype in both ATT determination. In electrophoretic profiles of proteins, while some HSPs were newly synthesized, some normal cellular proteins disappeared at 37 °C and 37 °C → 50 °C compared to 23 °C. The number of low molecular weight (LMW) HSPs were more than intermediate- (IMW) and high- (HMW) HSPs. The genotypes had both common (12 HSPs between at least two genotypes) and genotype-specific (33 HSPs) LMW HSPs. The common HSP of 19.8 kDa (pI 6.5) was synthesized in Bezostaya-1, Çukurova-86, Diyarbakır-81, *Ae. biuncialis* and *Ae. umbellulata*. Bezostaya-1 is the only genotype that synthesized 12 IMW and 2 HMW HSPs at 37 °C → 50 °C. *Ae. triuncialis* had only two common LMW HSPs [22.1 (pI 7.1) and 24.2 kDa (pI 6.5)].

**Keywords:** Wheat cultivars – *Aegilops* species – growth – cell viability – heat shock proteins

### INTRODUCTION

The productivity of wheat is limited by the genetic potential of the plant and the high temperature [32]. Plants acquire thermal tolerance to lethal high temperatures if first exposed to sublethal, acclimation temperatures or induction stress or if temperature is increased gradually to an otherwise lethal temperatures [2, 11, 16, 18, 27, 35]. The

\* Corresponding author; e-mail: mustafa\_yildizus@yahoo.com

growth of some monocotyledone and dicotyledone seedlings was shown to be protected during lethal temperature treatment by a sublethal temperature. Triphenyl tetrazolium chloride (TTC) cell viability assay is based on the principles of tetrazolium salt reduction to formazan by dehydrogenase respiratory enzymes. Therefore, TTC test evaluates the mitochondrial electron transport chain, and thus, it represents respirational activity. This assay has a long history of use as an estimate of plant cell and tissue viability at high temperatures [36]. Many researchers reported significant genetic differences in acquired thermal tolerance, and concluded that the TTC assay was efficient, reliable technique [9, 12, 30].

All the organisms synthesize heat shock proteins upon heat shock, but plants show a unique feature of heat shock response by synthesizing multiple families of small HSPs (LMW) in abundance [40]. Some HSPs themselves or their close relatives are present in all organisms at normal temperatures and play vital roles in normal cell function [19, 39]. HSPs are known to function as chaperones, playing an integral role in protein folding and assembly [39, 40]. A correlation between the development of thermal tolerance and the synthesis of HSP had been observed, although the mechanisms underlying the thermal tolerance are only beginning to be understood [4, 11, 20, 39].

The aim of this study is to evaluate the genotypic variability with use of growth analysis, cell viability assay (TTC) and heat shock protein patterns in seedlings obtained from cultivated and wild wheat genotypes differing in polyploidy level.

## MATERIALS AND METHODS

### *Plant materials and growth experiments*

Two bread wheat cultivars (*Triticum aestivum* L. cvs. Bezostaya-1, “B-1”, and Çukurova-86, “Ç-86”), one durum wheat (*Triticum durum* Desf. cv. Diyarbakır-81, “D-81”) and *Aegilops* L. species (tetraploid *Ae.biuncialis*, “*Ae. bi*”, and *Ae. triuncialis* “*Ae. tri*”; diploid *Ae. umbellulata*, “*Ae. umb*”) were used. Seeds of B-1, Ç-86 and D-81 were obtained from Eskehir, Çukurova and Southeast Anatolia Agricultural Research Institutes in Turkey, respectively. Ears of *Ae. bi* and *Ae. tri* were gathered from habitat of Southeast Anatolia (Şanlıurfa) in Turkey. Ears of *Ae. umb* were gathered from habitat of Central Anatolia (Kayseri) in Turkey. The selected and imbibed seeds were germinated at 23 °C and watered with 1/2 Hewitt nutrient solution when required. Two-day-old etiolated seedlings were exposed to different temperature treatments: 23 °C (C, control), 32 °C (T<sub>1</sub>), 35 °C (T<sub>2</sub>), 37 °C (T<sub>3</sub>) and 38 °C (T<sub>4</sub>) for 24 h, and T<sub>5</sub> (35 °C, 24 h → 50 °C, 1 h), T<sub>6</sub> (37 °C, 24 h → 50 °C, 1 h) and T<sub>7</sub> (23 °C, 24 h → 50 °C, 1 h). Then, the seedlings were transferred to 23 °C and sampled at the end of 48 and 72 h recovery growth periods. All experiments were carried out in dark-controlled different growth cabinets. The experiments were carried out with three replicates, each replicate consisted of four seedlings. At the end of each recovery growth period, the shoot length (SL, the part from the crown to the

tip of the first true leaf) was measured ( $\text{cm} \cdot \text{seedling}^{-1}$ ). The seedlings did not survive at  $23 \rightarrow 50^\circ\text{C}$  ( $T_7$ ). Acquired thermal tolerance (ATT) was calculated according to the ratio of shoot length measured at acclimation temperature treatments followed by heat shock treatments ( $T_5$  and  $T_6$ ) over their acclimation temperature treatments ( $T_2$  and  $T_3$ ) and it is formulated as below:

$$\text{ATT (\%)} = [\text{SL}_{T_5}/\text{SL}_{T_2}] \times 100 \text{ or } [\text{SL}_{T_6}/\text{SL}_{T_3}] \times 100$$

### *Cell viability (TTC reduction) test*

In the presence of viable tissue, the colorless solution of triphenyl tetrazolium chloride (TTC) is converted to the insoluble red triphenyl formazan [34]. One cm of coleoptile segments (3-day-old seedlings cut just over the crown) were used at the end of 24 h from  $23^\circ\text{C}$  (C, control),  $35^\circ\text{C}$  ( $T_2$ ) and  $37^\circ\text{C}$  ( $T_3$ ). Some of these coleoptile segments from C,  $T_2$  and  $T_3$  treatments were incubated in  $100\ \mu\text{L}$  distilled water at  $50^\circ\text{C}$  for 1 h [ $C_{(50)}$ ,  $T_{2(50)}$  and  $T_{3(50)}$ ] in boiling water for 1 minute [ $C_{(\text{boiled})}$ ,  $T_{2(\text{boiled})}$  and  $T_{3(\text{boiled})}$ ]. After incubations, 2 mL of TTC solution ( $8\ \text{mg mL}^{-1}$  TTC in  $0.05\ \text{M}$   $\text{K}_2\text{PO}_4$  buffer, pH 7.5 and  $0.5\ \text{mL L}^{-1}$  Tween 80) was immediately added into per tube, and infiltrated for approximately 10 min by vacuum. Then, coleoptile segments were removed and rinsed three times with distilled water. The formazan, which was produced by TTC reduction, was extracted with  $950\ \text{mL L}^{-1}$  ethanol. The level of formazan was assayed by reading the optical density (OD) at 475 nm with a double beam spectrophotometer (Jenway 6105 U.V./Vis.) using the procedure of Towill and Mazur [36] with minor modification.

Acquired thermal tolerance (ATT%) related with cell viability in the nonacclimated and acclimated coleoptile segments of etiolated seedlings is calculated by the following formula [30]:

$$\text{ATT\%} = \frac{\text{OD}[C]_{(50^\circ\text{C})} - \text{OD}[C]_{(\text{boiled})}}{\text{OD}[C]_{(23, 35 \text{ or } 37^\circ\text{C})} - \text{OD}[C]_{(\text{boiled})}} \times 100$$

$$\text{ATT\%} = \frac{\text{OD}[T_2]_{(50^\circ\text{C})} - \text{OD}[T_2]_{(\text{boiled})}}{\text{OD}[T_2]_{(23, 35 \text{ or } 37^\circ\text{C})} - \text{OD}[T_2]_{(\text{boiled})}} \times 100$$

$$\text{ATT\%} = \frac{\text{OD}[T_3]_{(50^\circ\text{C})} - \text{OD}[T_3]_{(\text{boiled})}}{\text{OD}[T_3]_{(23, 35 \text{ or } 37^\circ\text{C})} - \text{OD}[T_3]_{(\text{boiled})}} \times 100$$

### *Protein extraction and determination*

Two-day-old etiolated seedlings were set into three groups and each group was exposed to control ( $C=23^\circ\text{C}$ , 24 h), acclimation ( $T_3=37^\circ\text{C}$ , 24 h) or directly heat shock after acclimation ( $T_6=37^\circ\text{C}$ , 24 h  $\rightarrow 50^\circ\text{C}$ , 1 h) temperature treatments. Afterwards, the coleoptile tissues were frozen and ground in liquid nitrogen and total proteins extracted by addition of 5 mL of 10% trichloroacetic acid in acetone with 0.07%  $\beta$ -merkaptoethanol [8]. Protein concentrations of samples were determined by Bradford [3] and Ramagli and Rodriguez [31].

### *Protein electrophoresis*

The separation of normal and heat shock protein samples was performed by 2-DE. In the first dimension, isoelectric focusing was performed [26]. Isoelectric focusing gel solution was prepared with 10 g urea, 3 mL of 30% acrylamide-bisacrylamide, 0.2 mL ampholines (pH: 5–8), 0.8 mL ampholines (pH: 3–10), 0.1 mL Nonidet P-40 and 0.3 g CHAPS (Sigma, St. Louis, MO) and 7.4 mL distilled water [10]. Sample preparation and application for isoelectric focusing was carried out according to Naqvi et al. [24]. For electrophoresis in first dimension, the sample gels were prepared by mixing 50  $\mu$ L (containing 70  $\mu$ g soluble protein equivalent to BSA) of the supernatant with isoelectric focusing gel solution to make a total volume of 300  $\mu$ L. Then 1.5  $\mu$ L of 2.5% ammonium persulfate and 0.5  $\mu$ L of N,N,N',N'-tetramethylethylenediamine were added. A blank gel without protein sample was prepared in the same way for determination of the pH gradient. The cathodic solution was 20 mM sodium hydroxide while the anodic solution was 100 mM phosphoric acid. Run was performed with constant voltage of 400 volts for 3 hours followed by 800 volts for 17.15 hours. The second dimension sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was operated on 12% acrylamide gels according to the method of Laemmli [17]. 0.5% agarose with 0.01% bromphenol blue was layered on top of the gel. Commercial molecular weight markers (MW-SDS-70L) obtained from Sigma (St. Louis, MO) were prepared and used to standart. Separation for each slab gel was performed using 10 mA constant current for the first 30 minutes and then 25 mA for the rest of the run.

### *Gel staining and evaluation*

The gels were stained with silver dye [1] and air-dried [15]. Molecular weights ( $M_r$ , kDa) and isoelectric points ( $pI$ ) of heat shock proteins were determined.

### *Experimental design and statistical analysis*

The experiments were laid out in a randomized design. Differences between the temperature treatments as well as between the genotypes for all recovery periods in growth experiments and differences among the genotypes for cell viability assay data were tested using SPSS statistical programme. Statistical variance analysis of the independent data with twelve replicates ( $n = 12$ ) was performed using ANOVA and compared with least significant differences (LSD) at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

*Effect of different temperatures on shoot growth*

The shoot lengths of the genotypes generally decreased significantly with increasing temperature degrees except at 32 °C compared to 23 °C (control) (Fig. 1). This negative effect of the temperature was pronounced significantly for 48 and/or 72 h at 35 °C in *Ae. tri* and *Ae. umb*, at 37 °C in B-1, D-81, *Ae. bi*, *Ae. tri* and *Ae. umb*, and at 38 °C in all the genotypes. In contrast, the shoot length of cv. B-1 increased significantly at 35 °C for 48 h, while this significant increment disappeared at 72 h. The shoot length did not decrease significantly at 35 °C and 37 °C in cv. Ç-86, and at 38 °C in *Ae. tri* and *Ae. umb* compared to that previous temperatures for both recovery growth periods. At all temperatures, the shoot lengths of the genotypes differed significantly among themselves for 48 and 72 h. In this respect, the shoot length was significantly longer in *Ae. tri* and was significantly shorter in *Ae. umb* than in the other genotypes. The shoot growth was inhibited at 35, 37 and 38 °C temperature treatments, but similar genotypic differences were still found significant at these treatments.

Some seedlings were incubated at 23 °C, 35 °C and 37 °C for 24 h followed by heat shock (50 °C, 1 h) treatment: Severe heat girdling in the shoot was observed, the seedlings did not survive at 23 → 50 °C. On the other hand, the shoots were healthy at 35 → 50 °C and 37 → 50 °C, but their length decreased significantly at these treatments as compared to 35 and 37 °C for 48 and 72 h (Fig. 2). The shoot length of *Ae. tri* was longer than the other genotypes at control, 32, 35, 37 and 38 °C, but it was significantly shorter than cv. Ç-86 and cv. D-81 cultivars at 35 → 50 °C for 72 h, and cv. Ç-86 at 37 → 50 °C for 48 and 72 h. Also significant genotypic differences

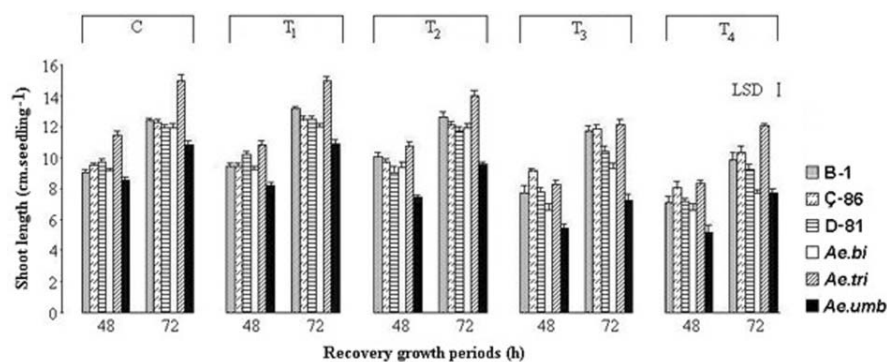


Fig. 1. Effect of different temperature treatments [C (control, 23 °C, 24 h), T<sub>1</sub> (32 °C, 24 h), T<sub>2</sub> (35 °C, 24 h), T<sub>3</sub> (37 °C, 24 h) and T<sub>4</sub> (38 °C, 24 h)] on the shoot lengths of wheat cultivars [Bezostaya-1 (B-1), Çukurova-86 (Ç-86) and Diyarbakır-81 (D-81)] and wild wheat species [*Ae. biuncialis* (*Ae. bi*), *Ae. triuncialis* (*Ae. tri*) and *Ae. umbellulata* (*Ae. umb*)]. Each mean is obtained from independent data with twelve replicates. Bars represent the standard error of means. LSD ( $P \leq 0.05$ )

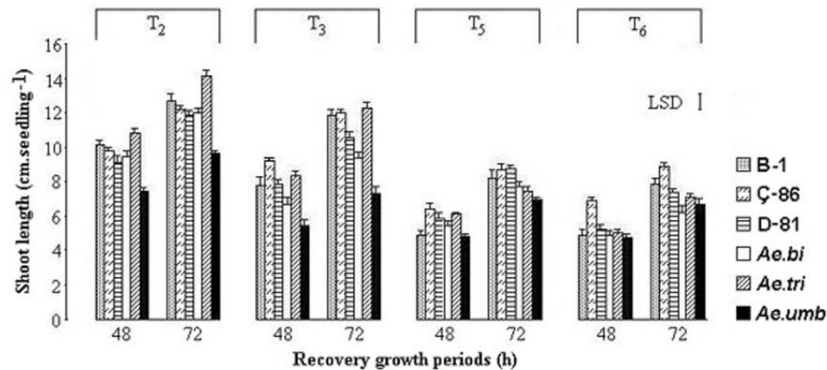


Fig. 2. Effect of different temperature treatments [T<sub>2</sub> (35 °C, 24 h), T<sub>3</sub> (37 °C, 24 h), T<sub>5</sub> (35 °C, 24 h → 50 °C, 1 h) and T<sub>6</sub> (37 °C, 24 h → 50 °C, 1 h)] on the shoot lengths of wheat cultivars [Bezostaya-1 (B-1), Çukurova-86 (Ç-86) and Diyarbakır-81 (D-81)] and wild wheat species [*Ae. biuncialis* (*Ae. bi*), *Ae. triuncialis* (*Ae. tri*) and *Ae. umbellulata* (*Ae. umb*)]. Each mean is obtained from independent data with twelve replicates. Bars represent the standard error of means. LSD ( $P \leq 0.05$ )

in the shoot lengths were observed between cv. B-1 and cv. Ç-86 at 37 → 50 °C as compared to 37 °C (Fig. 2). Temperatures 35 and 37 °C evoked significant levels of thermoprotection in the etiolated seedlings of the genotypes. Therefore, these temperatures could be accepted as acclimation temperatures.

Acquired thermal tolerance (ATT) was determined in percentage with respect to the shoot length (Fig. 3). ATT% of all genotypes was significantly high at the end of 48 h while ATT% of *Ae. umb* and *Ae. tri* was significantly high at the end of 72 h at

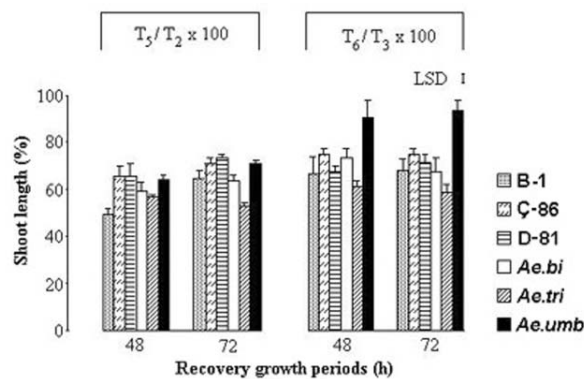


Fig. 3. Acquired thermal tolerance (%) of wheat cultivars [Bezostaya-1 (B-1), Çukurova-86 (Ç-86) and Diyarbakır-81 (D-81)] and wild wheat species [*Ae. biuncialis* (*Ae. bi*), *Ae. triuncialis* (*Ae. tri*) and *Ae. umbellulata* (*Ae. umb*)] calculated by the shoot lengths measured at acclimation temperature treatments [T<sub>2</sub> (35 °C, 24 h) and T<sub>3</sub> (37 °C, 24 h)] and each acclimation temperature treatment followed by heat shock treatment [T<sub>5</sub> (35 °C, 24 h → 50 °C, 1 h) and T<sub>6</sub> (37 °C, 24 h → 50 °C, 1 h)]. Each mean is obtained from independent data with twelve replicates. Bars represent the standard error of means. LSD ( $P \leq 0.05$ )

$T_6/T_3$  compared to  $T_5/T_2$ . The ratios of  $T_5/T_2$  (%) ranged from a high 74.08 in cv. D-81 to a low 53.17 in *Ae. tri*. The ratios of  $T_6/T_3$  (%) ranged from a high 93.52 in *Ae. umb* to a low 58.71 in *Ae. tri*.

Lin et al. [18] demonstrated that soybean seedlings develop thermal tolerance to an otherwise lethal temperature (2 h at 45 °C) by prior heat shock at an acclimation temperature (2 h at 40 °C). The sublethal shock at 39 °C has largely protected the coleoptiles of winter wheat cultivars against the subsequent lethal (45 °C) treatment [2]. Evidences suggest that genetic variability for thermal tolerance is seen only upon acclimation temperatures or induction stress [13, 16, 35, 37]. The results from our study also indicate that the seedlings, which were directly exposed to the lethal temperature ( $T_7 = 23$  °C, 24 h  $\rightarrow$  50 °C, 1 h), did not exhibit any genetic variability in thermal tolerance. On the contrary, the acclimated seedlings exhibited larger genetic variability for thermal tolerance.

The structural and functional changes such as synthesis of HSPs, accumulation of HSPs in pre-ribosome material of nucleolus, protection of cytoskeleton (microtubules and microfilament), polysomes, endoplasmic reticulum and dictyosome, formation of cytoplasmic aggregates (heat shock granules) at acclimation temperatures could be the result of thermal tolerance. Therefore, seedlings probably survived at acclimation temperatures followed by heat shock treatment. The structural and functional changes at 35 °C and 37 °C acclimation temperature treatments were probably similar. The acclimation temperature, 37 °C, is probably more effective than 35 °C in gaining ATT.

#### *Evaluation of cellular thermal tolerance using the TTC reduction test*

The results of the TTC reduction test are given in Table 1. At 23 °C treatment, there were some significant genotypic differences among the cultivars and the species. These significant genotypic differences in optical density were not observed at 23  $\rightarrow$  50 °C treatment. When values of 23  $\rightarrow$  50 °C treatment and boiled (1 min) coleoptile segments were put into the formula for determining ATT, the resulting values were negative. This clearly demonstrated that the coleoptile segments of nonacclimated etiolated seedlings were not capable to acquire thermal tolerance. This is consistent with reports on leaves of winter wheat cultivars [30], and thermotolerant and thermosensitive cultivars of bean, potato, soybean, tomato [5].

On the other hand, cellular ATT ranged from 2.75 to 32.87 at 35 °C and it ranged from 2.82 to 37.82 at 37 °C treatment in *Ae. tri*, *Ae. umb*, *Ae. bi*, D-81, Ç-86 and B-1, respectively (Table 1). There were significant differences among the cultivars and species at 35 and 37 °C treatments. OD values of the genotypes were found higher at 35 and 37 °C compared to 35  $\rightarrow$  50 °C and 37  $\rightarrow$  50 °C treatments. Genotypic variability of cultivars and species was increased significantly with acclimation followed by heat shock. B-1 was found to be a more thermotolerant genotype, while *Ae. tri* was determined as more thermosensitive. Although acclimated coleoptile segments

were partly injured from heat shock, their viability continued. Genotypic differences in cell viability were observed between the cultivars Mustang and Sturdy upon exposure to a lethal temperature of 50 °C, following a 17 h 37 °C heat shock treatment [14]. Leaf segments that had not been heat shocked prior to the 50 °C treatment exhibited a rapid decline in cell viability as determined by the TTC reduction assay. A complete loss in TTC reduction was observed within 60 min of the 50 °C treatment in the control tissues while reduction levels of 80 and 50% were retained in the

*Table 1*  
Acquired cellular thermal tolerance values of nonacclimated (23 °C) and acclimated (35 °C and 37 °C) wheat cultivars and wild wheat species measured by TTC reduction in coleoptile segments of etiolated seedlings

| Cultivars or species   | Nonacclimated                               |                              |                               | ATT %                       |
|------------------------|---------------------------------------------|------------------------------|-------------------------------|-----------------------------|
|                        | 23 °C                                       | 50 °C                        | boiled                        |                             |
|                        | OD 475                                      |                              |                               |                             |
| Bezostaya-1            | 3.803 <sup>a*</sup><br>±0.173 <sup>**</sup> | 0.064 <sup>a</sup><br>±0.008 | 0.214 <sup>a</sup><br>±0.015  | -4.18 <sup>***</sup>        |
| Çukurova-86            | 3.320 <sup>b</sup><br>±0.094                | 0.065 <sup>a</sup><br>±0.006 | 0.186 <sup>b</sup><br>±0.008  | -3.86                       |
| Diyarbakır-81          | 3.746 <sup>a</sup><br>±0.063                | 0.065 <sup>a</sup><br>±0.005 | 0.172 <sup>b</sup><br>±0.008  | -2.99                       |
| <i>Ae. biuncialis</i>  | 3.420 <sup>b</sup><br>±0.086                | 0.020 <sup>b</sup><br>±0.003 | 0.120 <sup>c</sup><br>±0.008  | -3.03                       |
| <i>Ae. triuncialis</i> | 2.178 <sup>c</sup><br>±0.066                | 0.014 <sup>b</sup><br>±0.006 | 0.074 <sup>d</sup><br>±0.005  | -2.85                       |
| <i>Ae. umbellulata</i> | 2.674 <sup>d</sup><br>±0.052                | 0.028 <sup>b</sup><br>±0.006 | 0.094 <sup>d</sup><br>±0.006  | -2.56                       |
| Acclimated (35 °C)     |                                             |                              |                               |                             |
|                        | 35 °C                                       | 50 °C                        | boiled                        | ATT %                       |
|                        | OD 475                                      |                              |                               |                             |
| Bezostaya-1            | 4.399 <sup>a</sup><br>±0.151                | 1.515 <sup>a</sup><br>±0.190 | 0.103 <sup>ab</sup><br>±0.007 | 32.87 <sup>a</sup><br>±3.89 |
| Çukurova-86            | 4.018 <sup>b</sup><br>±0.089                | 1.076 <sup>b</sup><br>±0.085 | 0.115 <sup>a</sup><br>±0.008  | 24.62 <sup>b</sup><br>±1.86 |
| Diyarbakır-81          | 4.275 <sup>ab</sup><br>±0.120               | 0.989 <sup>b</sup><br>±0.062 | 0.089 <sup>bc</sup><br>±0.007 | 21.50 <sup>b</sup><br>±1.71 |
| <i>Ae. biuncialis</i>  | 3.090 <sup>c</sup><br>±0.133                | 0.631 <sup>c</sup><br>±0.052 | 0.088 <sup>b</sup><br>±0.006  | 18.09 <sup>b</sup><br>±2.03 |
| <i>Ae. triuncialis</i> | 2.286 <sup>d</sup><br>±0.079                | 0.132 <sup>d</sup><br>±0.030 | 0.071 <sup>c</sup><br>±0.005  | 2.75 <sup>c</sup><br>±1.37  |
| <i>Ae. umbellulata</i> | 2.537 <sup>d</sup><br>±0.070                | 0.352 <sup>d</sup><br>±0.052 | 0.086 <sup>bc</sup><br>±0.007 | 10.85 <sup>d</sup><br>±1.94 |

Table 1 (cont.)

|                        | Acclimated (37 °C)           |                               |                               | ATT<br>%                    |
|------------------------|------------------------------|-------------------------------|-------------------------------|-----------------------------|
|                        | 37 °C                        | 50 °C                         | Boiled                        |                             |
|                        | OD 475                       |                               |                               |                             |
| Bezostaya-1            | 5.318 <sup>a</sup><br>±0.127 | 2.074 <sup>a</sup><br>±0.171  | 0.101 <sup>a</sup><br>±0.006  | 37.82 <sup>a</sup><br>±2.79 |
| Çukurova-86            | 3.778 <sup>b</sup><br>±0.328 | 1.201 <sup>bc</sup><br>±0.122 | 0.102 <sup>a</sup><br>±0.005  | 29.90 <sup>b</sup><br>±2.30 |
| Diyarbakır-81          | 4.679 <sup>c</sup><br>±0.132 | 1.316 <sup>b</sup><br>±0.049  | 0.069 <sup>b</sup><br>±0.004  | 27.05 <sup>b</sup><br>±1.20 |
| <i>Ae. biuncialis</i>  | 3.475 <sup>b</sup><br>±0.120 | 1.032 <sup>c</sup><br>±0.045  | 0.053 <sup>bc</sup><br>±0.007 | 28.61 <sup>b</sup><br>±2.15 |
| <i>Ae. triuncialis</i> | 2.389 <sup>d</sup><br>±0.107 | 0.113 <sup>d</sup><br>±0.023  | 0.047 <sup>c</sup><br>±0.005  | 2.82 <sup>c</sup><br>±1.03  |
| <i>Ae. umbellulata</i> | 2.685 <sup>d</sup><br>±0.096 | 0.404 <sup>e</sup><br>±0.043  | 0.098 <sup>a</sup><br>±0.006  | 11.83 <sup>d</sup><br>±1.73 |

\* Values followed by the same letters in each column are not significantly different according to the LSD ( $P \leq 0.05$ ).

\*\* Values represent the standart errors.

\*\*\* ATT% values in this column are not alphabeted because of negative.

leaf segments of the acclimated plus heat shocked Mustang and Sturdy, respectively. In addition, the dramatically different levels of thermal tolerance detected among different genotypes of bean, potato, soybean and tomato [5], winter and spring cultivars [9, 12, 30] after acclimation for 24 h at temperatures above 30 °C, is confirmed by our results. TTC reduction has been used as a cell viability test since 1951 year and represented a simple, rapid means of assessing viability when dealing with large numbers of samples.

### Synthesis of heat shock proteins

Newly synthesized heat shock proteins (LMW, 14.2–31.5 kDa; IMW, 31.6–62.5 kDa and HMW, 62.6–70 kDa) were classified and compared regarding molecular weights ( $M_r$ , kDa) and isoelectric points ( $pI$ ). At least three electrophoresis repeats were performed for each set of experiment and the proteins that exhibited similar behavior were included in the data.

At the heat shock treatments compared to control temperature, the synthesis of some normal cellular proteins disappeared in coleoptile tissues of the genotypes (C gels in Figs 4 and 5). Disappeared proteins have possibly become unstable as a consequence of heat shock treatments. On the other hand, newly synthesized HSPs in coleoptile tissues of wheat cultivars and wild wheat species ranged 15.7–67.9 kDa

*Table 2*  
Low molecular weight heat shock proteins synthesized in same molecular weight ( $M_r$ , kDa) and isoelectric point (pI) between at least two genotypes in coleoptile samples of *Triticum* cultivars and *Aegilops* species at acclimation ( $T_3 = 37^\circ\text{C}$ , 24 h) and directly heat shocked after acclimation ( $T_6 = 37^\circ\text{C}$ , 24 h  $\rightarrow 50^\circ\text{C}$ , 1h) or only  $T_6$  temperature treatments compared to control temperature ( $C = 23^\circ\text{C}$ , 24 h) treatment

| Triticum cultivars     |             |                |                |                |                |                |                |                |                |                |                | Aegilops species |                |                |                |                |                |  |  |         |  |  |  |
|------------------------|-------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|------------------|----------------|----------------|----------------|----------------|----------------|--|--|---------|--|--|--|
| B-1                    |             |                |                | Ç-86           |                |                |                | D-81           |                |                |                | Ae. bi           |                |                |                | Ae. tri        |                |  |  | Ae. umb |  |  |  |
| Temperature treatments |             |                |                |                |                |                |                |                |                |                |                |                  |                |                |                |                |                |  |  |         |  |  |  |
| Spot No.               | Mr-pI       | T <sub>3</sub> | T <sub>6</sub> | T <sub>3</sub> | T <sub>6</sub> | T <sub>3</sub> | T <sub>6</sub> | T <sub>3</sub> | T <sub>6</sub> | T <sub>3</sub> | T <sub>6</sub> | T <sub>3</sub>   | T <sub>6</sub> | T <sub>3</sub> | T <sub>6</sub> | T <sub>3</sub> | T <sub>6</sub> |  |  |         |  |  |  |
| 1                      | 19.8 (6.5)  | +              | +              | +              | +              | +              | +              | +              | +              | +              | +              | +                | +              | +              | +              | +              | +              |  |  |         |  |  |  |
| 2                      | 20.1 (<5.2) |                |                |                |                |                |                |                |                |                |                |                  |                | +              | +              |                | +              |  |  |         |  |  |  |
| 3                      | 20.7 (6.7)  | +              | +              | +              | +              | +              | +              | +              | +              | +              | +              | +                | +              |                |                | +              | +              |  |  |         |  |  |  |
| 4                      | 20.7 (7.1)  | +              | +              | +              | +              | +              | +              | +              | +              | +              | +              | +                | +              |                |                | +              | +              |  |  |         |  |  |  |
| 5                      | 21.2 (6.9)  | +              | +              | +              | +              | +              | +              | +              | +              | +              | +              | +                | +              |                |                |                |                |  |  |         |  |  |  |
| 6                      | 22.1 (7.1)  |                |                |                |                |                |                |                |                |                |                |                  |                | +              | +              |                |                |  |  |         |  |  |  |
| 7                      | 24.0 (<5.2) |                |                |                |                |                |                |                |                |                |                | +                | +              |                |                | +              | +              |  |  |         |  |  |  |
| 8                      | 24.2 (6.5)  |                |                |                |                |                |                |                |                |                |                | +                | +              | +              | +              |                |                |  |  |         |  |  |  |
| 9                      | 24.8 (<5.2) |                |                |                |                |                |                |                |                |                |                | +                | +              | +              | +              | +              | +              |  |  |         |  |  |  |
| 10                     | 26.9 (6.0)  |                |                |                |                |                |                |                |                |                |                | +                | +              | +              | +              |                |                |  |  |         |  |  |  |
| 11                     | 27.0 (6.1)  |                |                |                |                |                |                |                |                |                |                | +                | +              | +              | +              |                |                |  |  |         |  |  |  |
| 12                     | 27.2 (6.0)  |                |                |                |                |                |                |                |                |                |                | +                | +              | +              | +              |                |                |  |  |         |  |  |  |

(pH is generally acidic) at 37 °C for 24 h ( $T_3$ ) and 37 °C for 24 h plus 50 °C for 1 h ( $T_6$ ) or only  $T_6$  temperature treatments compared to control temperature (C, 23 °C for 24 h) treatment.

In the coleoptile tissues of the genotypes, commonly synthesized LMW HSPs (1–12 protein spots) between at least two genotypes were shown in Table 2, Figs 4 and 5. These common LMW HSPs were ranged from 19.8 to 27.2 kDa ( $pI$  <5.2–7.1). The common HSP of 19.8 kDa ( $pI$  6.5) was synthesized in Bezostaya-1, Çukurova-86, Diyarbakır-81, *Ae. biuncialis* and *Ae. umbellulata*. B-1 and Ç-86 cultivars have newly synthesized four common polypeptides (spots no. 1, 3, 4 and 5 in Table 2, Figs 4 and 5). *Ae. triuncialis* had only two common LMW HSPs [22.1 ( $pI$  7.1) and 24.2 kDa ( $pI$  6.5)]. These results show that species or cultivars derived from different genomes can synthesize similar polypeptides. Similar results were also reported by Ouerghi et al. [28].

HSPs synthesized at high temperature treatments showed genetic variability. Some HSPs were specific to the genotype(s). In cv. D-81 and *Ae. umb*, some LMW HSPs observed at  $T_3$  were also determined at  $T_6$  temperature treatment. Total 67 LMW HSPs were synthesized in all genotypes at  $T_3$  and  $T_6$  compared to control. The 12 IMW [32.5 (3 protein spots in  $pI$  5.3–5.6), 38.6 ( $pI$  7.7), 41.1 ( $pI$  6.3), 42.9 ( $pI$  6.3), 51.6 ( $pI$  6.1; 6.2), 52.9 ( $pI$  6.2), 56.2 ( $pI$  6.0), 57.5 ( $pI$  6.0) and 58.8 kDa ( $pI$  5.1)] and 2 HMW [67.9 kDa (2 protein spots in  $pI$  5.7 and 5.8)] HSPs were only synthesized in coleoptile tissue of cv. B-1 ( $T_6$  gel in Fig. 4). At  $T_6$  temperature treatment, 10 LMW HSPs (with asterisk and without asterisk) synthesized at high temperature treatments were only determined in cv. D-81, while 25.5 kDa ( $pI$  6.6) was only synthesized in cv. Ç-86 (Fig. 4). On the other hand, IMW HSPs were synthesized in cv. Ç-86 [45.0 (3 protein spots in  $pI$  6.0–6.1) and 50.3 kDa (3 protein spots in  $pI$  6.0–6.1)] and *Ae. bi* [39.9 kDa ( $pI$  5.8–6.0)] (Figs 4 and 5). In addition, HMW HSPs [66.3, 66.5 and 66.7 kDa ( $pI$  6.1)] were only determined in cv. D-81 at  $T_3$  temperature treatment (Fig. 4). Three IMW [34.4–41.6 kDa ( $pI$  7.5–7.7)] and two LMW [27.6 and 27.8 kDa ( $pI$  6.2)] HSPs were specific to *Ae. tri* at  $T_3$  temperature treatment (Fig. 5). The LMW HSPs (denoted as proteins in Fig. 5) of 17.1, 18.6, 21.8 ( $pI$  <5.2), 19.8 ( $pI$  6.7), 21.8 ( $pI$  6.4 and 6.5) and 23.4 kDa ( $pI$  6.4) synthesized at both high temperature treatments were specific to *Ae. umb*. The physiological significance of these HSPs is unknown and it would be interesting to determine whether they play a thermoprotective role. HSP diversity, along with known diversity of thermal tolerance, allows an analysis of specific, unique HSPs with respect to thermal tolerance. On the other hand, Cooper and Ho [6] determined that the 70 kDa HMW HSP had conserved as evolutionary in maize. Krishnan et al. [14] reported that the genotypic differences in HSPs synthesis in thermo-tolerant ‘Mustang’ and thermo-sensitive ‘Sturdy’ winter wheat varieties were also determined. It has been suggested that HSPs 68, 21 and 17 which present in the structure of HSP granules are effected in the protection of mRNA [22] and HSP 15–18 which localized in plasma membrane is effected in the protection of membrane permeability [7].

The appearance of HSPs in coleoptile tissues is associated with a concomitant reduction or disappearance in normal protein synthesis while the exact synthesis of

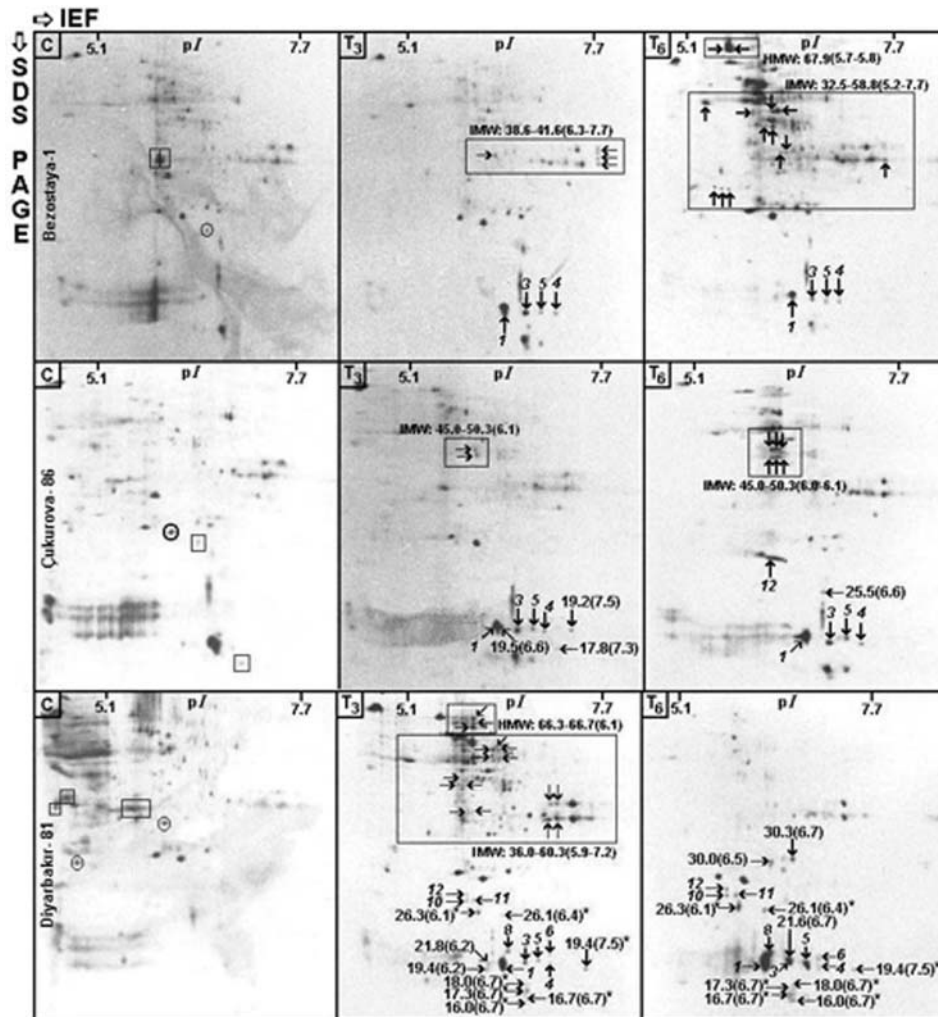


Fig. 4. 2-D patterns of heat shock proteins (HSPs) extracted from coleoptile tissues of wheat cultivars (Bezostaya-1, Çukurova-86 and Diyarbakır-81) at control (C = 23 °C, 24 h), acclimation (T<sub>3</sub> = 37 °C, 24 h) and heat shocked after acclimation (T<sub>6</sub> = 37 °C, 24 h → 50 °C, 1 h) temperature treatments. The disappeared normal cellular proteins at T<sub>3</sub> and T<sub>6</sub> temperature treatments are shown by circles (disappeared at only T<sub>6</sub>) and squares (disappeared at both T<sub>3</sub> and T<sub>6</sub>) on control (C) gels. The HSPs are indicated by arrows. The numbers (1–12) indicate the low molecular weight (LMW) HSPs synthesized between at least two genotypes (see Table 2). The decimal numbers [molecular weight (Mr, kDa) and isoelectric point (pI), respectively] without asterisk indicate the LMW HSPs synthesized at only a temperature treatment within each genotype, while the others with asterisk indicate the LMW HSPs synthesized at T<sub>3</sub> and T<sub>6</sub> temperature treatments within each genotype. The intermediate (IMW) and high molecular weight (HMW) HSPs are shown by arrows in boxes

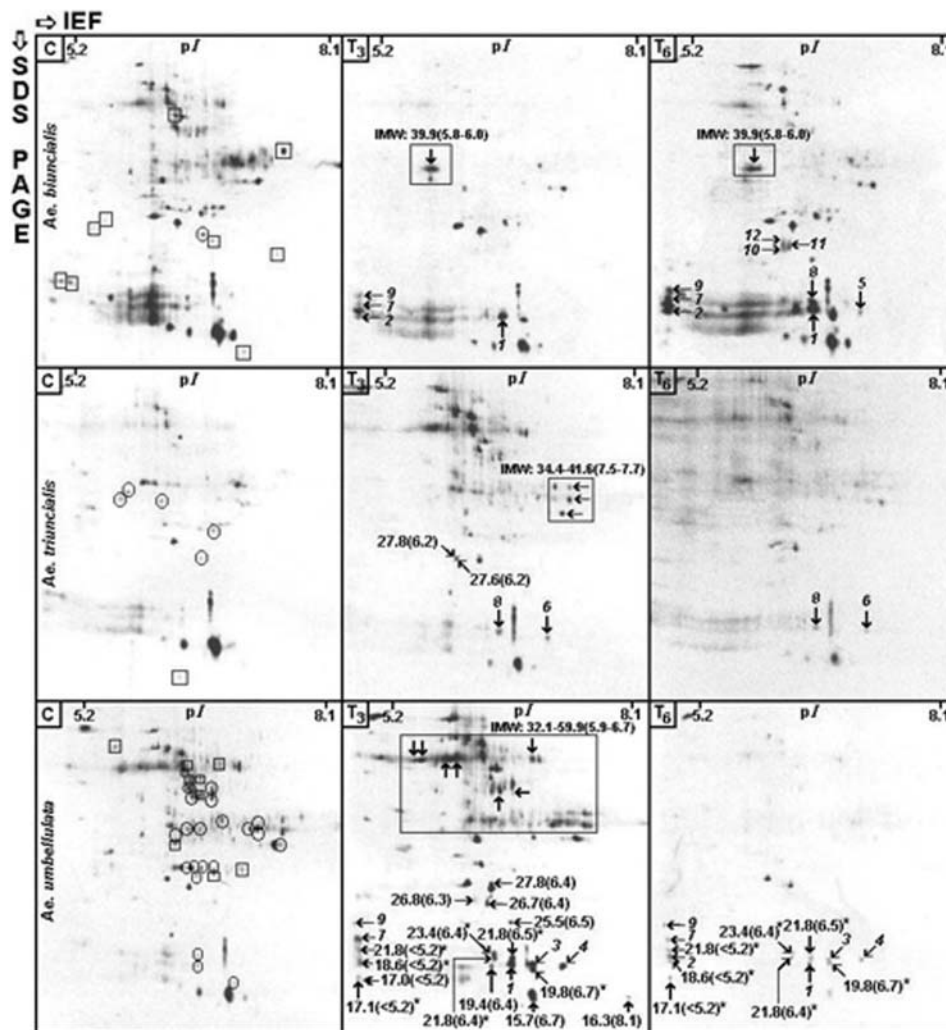


Fig. 5. 2-D patterns of heat shock proteins (HSPs) extracted from coleoptile tissues of wild wheat species (*Ae. biuncialis*, *Ae. triuncialis* and *Ae. umbellulata*) at control (C = 23 °C, 24 h), acclimation (T<sub>3</sub> = 37 °C, 24 h) and heat shocked after acclimation (T<sub>6</sub> = 37 °C, 24 h → 50 °C, 1 h) temperature treatments. The disappeared normal cellular proteins at T<sub>3</sub> and T<sub>6</sub> temperature treatments are shown by circles (disappeared at only T<sub>6</sub>) and squares (disappeared at both T<sub>3</sub> and T<sub>6</sub>) on control (C) gels. The HSPs are indicated by arrows. The numbers (1–12) indicate the low molecular weight (LMW) HSPs synthesized between at least two genotypes (see Table 2). The decimal numbers [molecular weight (Mr, kDa) and isoelectric point (pI), respectively] without asterisk indicate the LMW HSPs synthesized at only a temperature treatment within each genotype, while the others with asterisk indicate the LMW HSPs synthesized at T<sub>3</sub> and T<sub>6</sub> temperature treatments within each genotype. The intermediate (IMW) HSPs are shown by arrows in boxes

normal cellular proteins were shown. The similar results reported in maize [6], wheat, pea, rice, sunflower, sorghum, soybean [21] and wheat [14, 33].

In this study, the number of LMW HSPs was more than IMW and HMW HSPs. Some LMW HSPs were accumulated to big spots. These spots show up that LMW HSPs have complex structure. Waters et al. [40] reported that small heat shock proteins (sHSPs = LMW) synthesized in higher plants were more and different than other eukaryotic. Genetic variability in HSPs have identified in wheat cultivars that synthesized in hexaploid [14, 25, 33, 42], tetraploid [25] and diploid [38] cultivars. In our research, HSPs showed genetic variability in cultivated and wild wheat genotypes which are in genetic relationship. These proteins might have possibly function in acquisition of thermal tolerance. HSPs are chaperons, which function during normal cell growth and stress conditions [23]. This interaction was determined with one-and/or two-dimension SDS-PAGE technique in proteins of total tissues or specificity to organelles in different plant genotypes exposed to different temperature treatments [18, 21, 33, 38].

The results of this study showed that acquired thermotolerance of tetraploid and hexaploid wheat cultivars was higher than in diploid and tetraploid wild wheat species in early seedling stage which is most thermosensitive. Genotypic differences in tolerance to high temperature showed that thermal tolerance and HSPs synthesis capacity depended on changes at degrees and periods of temperature [29]. Krishnan et al. [14] suggested a positive interaction between the genotypic differences in relation to LMW HSPs synthesis levels and thermal tolerance. In addition, the reason and effect relationship of data is not to be discussed. Also Krishnan et al. [14] reported that LMW HSPs have adaptive role at thermal tolerance. Xiao and Mascarenhas [41] reported that HSPs were not synthesized in pollen tubes of *Tradescantia paludosa*. As described above, HSPs may be very important to thermal tolerance induction.

According to our findings, early HSPs were synthesized at acclimation temperature ( $T_3$ ) while late HSPs were synthesized at acclimation temperature followed by heat shock ( $T_6$ ). Necchi et al. [25] reported that gradually synthesis of new HSPs was described in the coleoptiles of some monocotyledons at 40 °C for 4, 7 and 12 h.

Development of heat-tolerant wheat cultivars and wild species is of major concern in wheat breeding programs. A detailed understanding of the genetics and physiology of heat tolerance as well as the use of the proper germplasm and selection methods will facilitate the development of heat tolerant cultivars of wheat. The genetic modification of plants to grow and produce an economic yield under the given environmental constraints remains the most feasible solution to problems of environmental stress. Two main routes to increase heat tolerance are selection and genetic transfer. Selection does not only mean looking for a high yielding genotype under a given set of environmental conditions but it also means identification of a potential source for genetic material to be used in breeding or transfer.

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