

SYNTHESIS OF SOLUBLE HEAT SHOCK PROTEINS IN SEMINAL ROOT TISSUES OF SOME CULTIVATED AND WILD WHEAT GENOTYPES

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Effect of heat stress on the synthesis of soluble heat shock proteins (HSPs) and the regrowth in seminal roots of three cultivated and three wild wheat genotypes was examined. In regrowth experiments, 2-d-old etiolated seedlings were exposed to 23 (control), 32, 35, 37 and 38 °C for 24 h, and 35 and 37 °C (24 h) followed by 50 °C (1 h). The lengths of the seminal roots generally decreased significantly at the end of 48 and 72 h recovery growth periods at 35, 37 and 38 °C temperature treatments compared with control. Genotypic variability was significant level at all temperature treatments for the seminal root length. Also, genotypic differences for the number of seminal roots were determined among the wheat cultivars and between the wild wheat species and the wheat cultivars at all temperature treatments; but genotypic differences among wild wheat species were only detected at 37→50 °C treatment. Acquired thermotolerance for the seminal root length is over 50% at 37→50 °C treatment. The genotypic variability of soluble heat shock proteins in seminal root tissues were analyzed by two-dimensional electrophoresis (2-DE). Total number of low molecular weight (LMW) HSPs was more than intermediate- (IMW) and high- (HMW) HSPs at high temperature treatments. The most of LMW HSPs which were generally of acidic character ranged between 14.2–30.7 kDa. The genotypes had both common (43 HSP spots between at least two genotypes and 23 HSP spots between 37 and 37 → 50 °C) and genotype-specific (72 HSP spots) LMW HSPs.

Keywords: *Triticum* – *Aegilops* – seminal root – regrowth – heat shock proteins

INTRODUCTION

Heat stress is one of the most important factors affecting crop yield [32]. A small increase above optimal growth temperature has a large effect on growth rate of plants [9]. Temperature affects development and the duration of various developmental stages. In addition, high temperatures have deleterious effects on several physiolog-

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ical processes like respiration, reproduction and in particular, seedling survival [27]. Plant regrowth, although a sensitive assay for heat tolerance, is limited by the length of time required to detect an effect [33]. Plants cannot avoid stress conditions when the temperature of environment is rising to sublethal or even lethal levels. A short pre-exposition of plants to temperatures close to the lethal temperature leads to an enhanced thermoresistance. This higher level of induced heat resistance is called acquired thermotolerance [30]. In response to heat stress the plant cell starts to synthesize a set of special proteins called heat shock proteins (HSPs) [31]. All HSPs create the complex functional network of the heat shock response that enables the plant to survive elevated temperatures [30]. Plants synthesize lower molecular weight (LMW) HSPs than other organisms [12]. The HSPs in elongating and mature sections of primary roots of 3-d-old maize seedlings are synthesized at 40 °C [6]. The synthesis of several LMW HSPs has been suppressed at 40 °C in the hybrid 222 corn.

In this study, the regrowth and the acquired thermotolerance of the seminal roots of cultivated and wild wheats after different heat treatments was determined with regrowth experiments. The genotypic variability of HSPs was compared in the seminal root tissues of cultivated and wild wheats at 23 and 37 °C for 24 h, and at 37 °C (24 h) followed by 50 °C (1 h) temperature treatments.

MATERIALS AND METHODS

Plant materials and treatments

Two common bread wheat (*Triticum aestivum* L., cv. Bezostaya-1 “B-1” and cv. Çukurova-86 “Ç-86”), one durum wheat (*Triticum turgidum* L. ssp. *durum* Desf., cv. Diyarbakır-81 “D-81”) and three wild wheat (*Aegilops* L. species, *Ae. biuncialis* “*Ae. bi*”, *Ae. triuncialis* “*Ae. tri*” and *Ae. umbellulata* “*Ae. umb*”) genotypes were studied. The B-1, Ç-86 and D-81 seeds were obtained from Eskişehir, Çukurova and South East Anatolia Agricultural Research Institutes in Turkey, respectively. The ears of *Ae. bi* and *Ae. tri* were gathered from habitat in South East Anatolia (Şanlıurfa) in Turkey. Ears of *Ae. umb* were gathered from habitat in Central Anatolia (Kayseri) in Turkey. The selected and imbibed seeds were germinated on moist filter paper (with distilled water) in germination cups in a controlled growth chamber (dark, 23 °C, 50–60% humidity) for two days. The seedlings were watered with half-strength Hewitt’s solution when required.

Regrowth experiments

After germination at 23 °C for 2 days, the etiolated seedlings were separated into 8 groups. Each group from cultivars/species was exposed to various temperature treatments: 23 (C, control), 32 (T₁), 35 (T₂), 37 (T₃) and 38 °C (T₄) for 24 h, and T₅ (35 °C, 24 h → 50 °C, 1 h), T₆ (37 °C, 24 h → 50 °C, 1 h) and T₇ (23 °C, 24 h → 50 °C,

1 h). Then, the etiolated seedlings were transferred to 23 °C and sampled at the end of 48 and 72 h recovery growth periods. The experiments were carried out with three replicates; each replicate consisted of four seedlings. At the end of each recovery period, the seminal root length (SRL, the part from the crown to the tip of the most longest seminal root axis) was measured ($\text{cm} \cdot \text{seedling}^{-1}$) and the counted number of seminal roots was expressed as $\sqrt{x} \cdot \text{seedling}^{-1}$. The seedlings did not survive at 23 → 50 °C (T_7). Therefore, the number and lengths of seminal roots were not determined at this treatment.

Acquired thermal tolerance (ATT) was calculated according to the ratio of seminal root 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:

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

Experimental design and statistical analysis

The experiments were laid out in a randomized design. The seminal root number data were square root transformed before statistical analysis. Differences between the temperature treatments as well as between the genotypes for all recovery periods in growth experiments 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 $P \leq 0.05$.

Protein extraction and determination

Intact 2-d-old seedlings were set into three groups and each group was exposed to control ($C = 23$ °C, 24 h), acclimation ($T_3 = 37$ °C, 24 h), and acclimation following by 50 °C for 1 h ($T_6 = 37$ °C, 24 h → 50 °C, 1 h) treatments. In brief, the seminal root tissues were frozen and ground in liquid nitrogen and total soluble proteins extracted by addition of 5 mL of 10% trichloroacetic acid in acetone with 0.07% β -mercaptoethanol [7]. Protein concentrations of samples were determined by Bradford [2] and Ramagli and Rodriguez [25].

2-D electrophoresis of soluble proteins

The separation of normal and heat shock protein samples was performed by 2-DE. In the first dimension, isoelectric focusing was performed [23]. Isoelectric focusing gel solution was prepared with 10 g urea, 7.4 mL distilled H_2O , 3 mL 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 3-[(3-Cholamidopropyl) dimethyl-ammonio]-1-propane-

sulfonate (CHAPS) [8]. Sample preparation and application for isoelectric focusing was carried out according to Naqvi et al. [20]. For electrophoresis in first dimension, the sample gels were prepared by mixing 50 μ L (containing 70 μ g soluble protein equivalent to BSA) of the supernatant with isoelectric focusing gel solution to make a total volume of 300 μ L. Then 1.5 μ L of 2.5% ammonium persulfate (APS) and 0.5 μ L of N,N,N',N'-tetramethylethylenediamine (TEMED) were added. Afterwards, the contents were loaded to glass capillary tubes with 1.5 mm inner diameter and 150 mm length. A blank gel without protein sample was prepared in the same way for determination of the pH gradient. As for pH gradient, blank gel was sliced into 1 cm pieces, each piece was transferred to 1.4 ml of 1 M KCl solution, kept overnight at 4 °C, and thence the pH was measured at 25 °C. The cathodic solution was 0.02 M sodium hydroxide while the anodic solution was 0.01 M phosphoric acid. Run was performed with constant voltage of 400 volts for 3 hours followed by 800 volts for 17.15 hours. In the second dimension, sodium dodecyl sulfate polyacrylamide gel electrophoresis on 12% acrylamide gels was performed [16]. 0.5% agarose with 0.01% bromphenol blue was layered on top of the gel. Isoelectric focusing gels were loaded on slab gels and separation was performed at 10 °C using 10 mA constant current per gel for the first 30 minutes and then 25 mA per gel for the rest of the run. Commercial molecular weight markers (10–70 kDa) were prepared and used to standard.

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.

RESULTS AND DISCUSSION

Regrowth in the seminal roots

The seminal root lengths of the genotypes generally decreased significantly at increasing temperature degrees compared to control except at 32 °C (Fig. 1A). Also, this effect was significant except in *Ae. umb* at 35 $\rightarrow$ 50 °C and 37 $\rightarrow$ 50 °C compared to 35 and 37 °C treatments at the end of 48 and 72 h (Fig. 1B). Genotypic differences for the seminal root lengths were significant between cultivars and species (cultivars were longer than species) and within them at control. These distinct differences generally disappeared at high temperature treatments (Fig. 1A and B). The number of the seminal roots increased by generation of new seminal roots (Fig. 2A and B): This effect was significant in cv. D-81 at 32 °C, 35 °C and 38 °C compared to control treatment at the end of 72 h. Also, this effect was significant in cv. B-1 at 37 $\rightarrow$ 50 °C compared to 37 °C treatment at the end of both recovery growth periods, and in *Ae. tri* and *Ae. umb* at 35 $\rightarrow$ 50 °C and 37 $\rightarrow$ 50 °C compared to 35 and 37 °C treatments at the end of 72 h (Fig. 2B). Typically, wheat produces three to six semi-

nal roots per plant and seminal root number is under genetic control [24]. Huang et al. [10] reported that the seminal root number is modified with temperature. In our study, the formation of new seminal root at $37 \rightarrow 50^\circ\text{C}$ treatment proved the genotypic differences among wild wheat species. Similar results were reported by Huang et al. [11].

All the genotypes showed a significantly different thermotolerance at 23, 35 and 37°C for 24 h followed by heat shock (50°C , 1 h) treatment (T_7 , T_5 and T_6 , respectively): Severe heat girdling in the seminal root axes were observed and the seedlings did not survive at T_7 treatment. On the other hand, the seedlings survived because the seminal roots were slightly damaged at T_5 and T_6 treatments, but the length of seminal root decreased significantly at these treatments compared with 35 and 37°C for 48 and 72 h. 35 and 37°C temperatures caused the roots to develop significant levels of thermoprotection. Therefore, these temperatures could be accepted as accli-

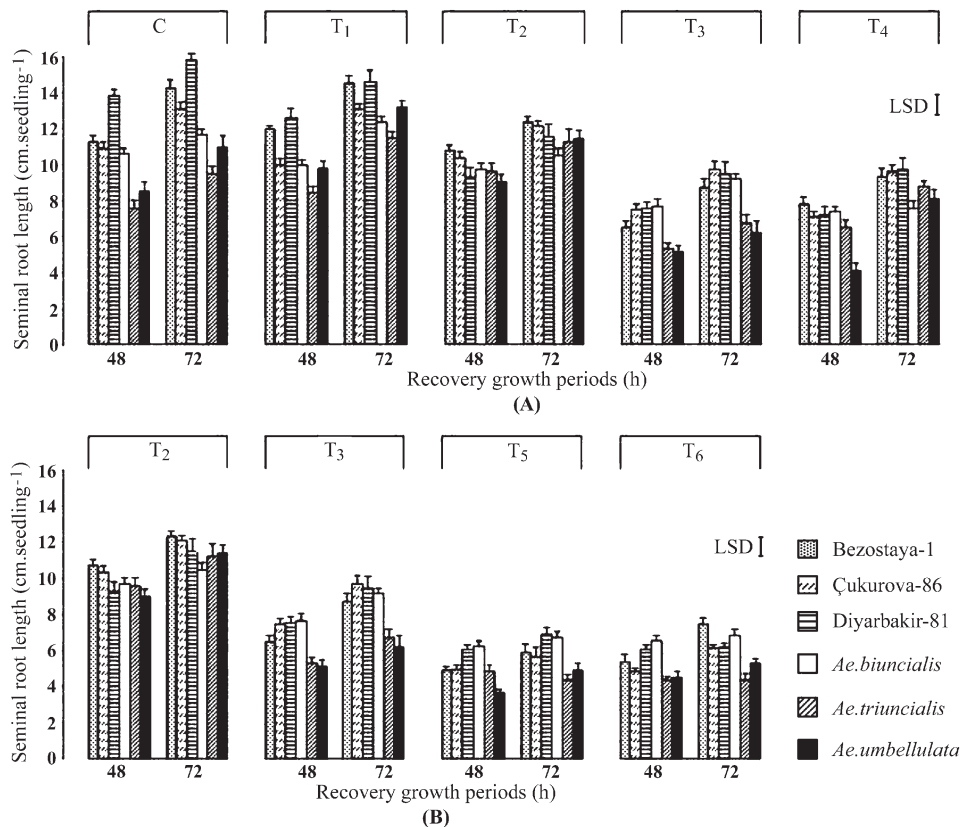


Fig. 1. Effect of different temperature treatments on the seminal root lengths of *Triticum* cultivars and *Aegilops* species. Temperature treatments: (A) C (23°C , 24 h), T_1 (32°C , 24 h), T_2 (35°C , 24 h), T_3 (37°C , 24 h) and T_4 (38°C , 24 h), and (B) T_2 , T_3 , T_5 (35°C , 24 h $\rightarrow$ 50°C , 1 h) and T_6 (37°C , 24 h $\rightarrow$ 50°C , 1 h). Each means obtained from the independent data with twelve replicates. Bars represent the standard error means. LSD ($P \leq 0.05$)

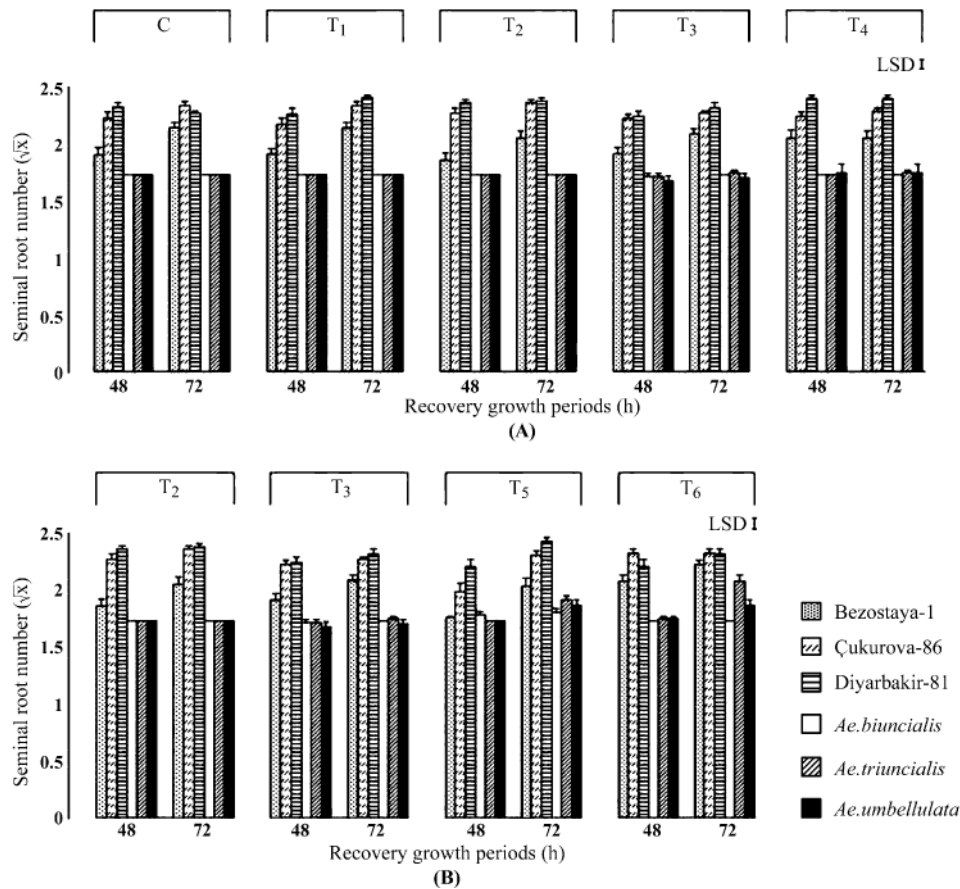


Fig. 2. Effect of different temperature treatments on the seminal root number of *Triticum* cultivars and *Aegilops* species. Temperature treatments: (A) C (23 °C, 24 h), T₁ (32 °C, 24 h), T₂ (35 °C, 24 h), T₃ (37 °C, 24 h) and T₄ (38 °C, 24 h), and (B) T₂, T₃, T₅ (35 °C, 24 h → 50 °C, 1 h) and T₆ (37 °C, 24 h → 50 °C, 1 h). Each means obtained from the independent data with twelve replicates. Bars represent the standard error means. LSD ($P \leq 0.05$)

mation temperatures. Chen et al. [4] reported that the acclimation mechanism may only work within a narrow temperature range. In their study, the temperature for maximum acclimation in the tested crops is detected to be about 35 to 37 °C.

Genotypic differences in acquired thermotolerance for the seminal root lengths were determined at T₅/T₂ and T₆/T₃ in % ratios (Fig. 3): ATT% of all the genotypes increased significantly at T₆/T₃ compared to T₅/T₂ at the end of both recovery growth periods. This result showed that the growth of seminal root at heat shock (50 °C, 1 h) was protected significantly by 37 °C pre-treatment. The acquired thermotolerance of only *Ae. tri* at T₅/T₂ was decreased significantly while the acquired thermotolerance of cv. D-81, *Ae. bi* and *Ae. tri* were diminished significantly at T₆/T₃ at the end of

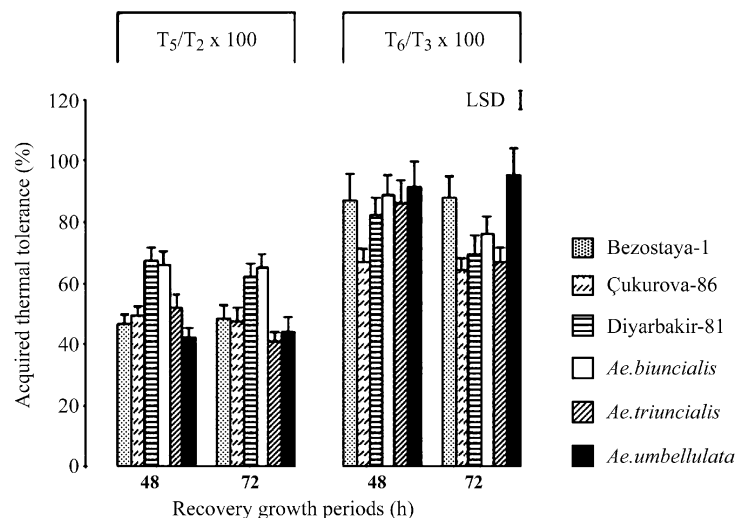


Fig. 3. Acquired thermal tolerance (%) of *Triticum* cultivars and *Aegilops* species. Each means obtained from the independent data with twelve replicates. Bars represent the standard error means. LSD ($P \leq 0.05$)

72 h compared to 48 h. *Ae. tri* was much more thermosensitive than the other genotypes at T_5/T_2 , in contrast *Ae. umb* was significantly more thermotolerant than the other genotypes except cv. B-1 at T_6/T_3 . The ratios of T_5/T_2 (%) ranged from a high 65.02 in *Ae. bi* to a low 40.74 in *Ae. tri*. The ratios of T_6/T_3 (%) ranged from a high 95.02 in *Ae. umb* to a low 64.19 in Ç-86. The acclimation temperature, 37 °C is probably more effective in gaining ATT than 35 °C.

Soluble heat shock proteins in seminal root tissues

Newly synthesized HSPs were determined in the seminal root tissues at acclimation temperature ($T_3 = 37$ °C, 24 h) and/or acclimation temperature following by heat shock temperature ($T_6 = 37$ °C, 24 h $\rightarrow$ 50 °C, 1 h) compared to the control ($C = 23$ °C, 24 h) temperature treatment. HSPs are classed and defined in terms of their approximate molecular weights in kDa: These are high mol weight (HMW) in the 110 to 65 kDa range [13, 18], intermediate mol weight (IMW) in the 62 to 32 kDa range [21], and low mol weight (LMW) about 30 to 15 kDa [31] in size. In our study, HSPs are classed as LMW between 14.2–31.5 kDa, IMW between 31.6–62.5 kDa and HMW between 62.6–70 kDa.

In this study, the number of LMW HSPs (275 spots) of all genotypes was more than IMW (98 spots) and HMW (23 spots) HSPs (Fig. 4a–c). Some LMW HSPs were accumulated into big spots. These spots show that LMW HSPs have complex structure. Waters et al. [31] reported that small heat shock proteins (sHSPs = LMW) syn-

Table 1

Synthesized low molecular weight heat shock proteins in same molecular weight (M_r , kDa) and isoelectric point (pI) between at least two genotypes in seminal root tissues 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 $\rightarrow 50^\circ\text{C}$, 1 h) or only T_6 temperature treatments compared to control temperature ($C = 23^\circ\text{C}$, 24 h) treatment

Spot No.	$M_r - pI$	Genotypes											
		Temperature treatments											
		Bezostaya-1		Çukurova-86		Diyarbakır-81		<i>Ae. biuncialis</i>		<i>Ae. triuncialis</i>		<i>Ae. umbellulata</i>	
		T_3	T_6	T_3	T_6	T_3	T_6	T_3	T_6	T_3	T_6	T_3	T_6
1	14.2 (6.3)	+	+		+						+		
2	14.2 (6.5)	+	+		+		+						
3	15.2 (5.7)	+	+		+						+		
4	15.5 (≤ 5.0)				+				+				
5	15.5 (7.2)				+		+						
6	16.5 (6.9)		+				+						
7	17.8 (7.6)						+				+		
8	18.3 (6.2)								+		+		
9	18.6 (6.6)	+	+								+		
10	19.2 (7.1)		+	+	+	+	+						
11	19.3 (6.1)	+	+	+	+	+	+				+	+	+
12	20.1 (6.3)	+	+	+	+			+	+	+	+	+	+
13	20.1 (6.8)	+	+	+	+	+	+	+	+		+		
14	20.1 (7.0)	+	+	+	+	+	+	+	+	+	+	+	+
15	21.1 (6.9)	+	+	+	+								
16	21.1 (7.2)						+				+		
17	22.1 (6.5)	+	+	+	+	+	+	+	+	+	+	+	+
18	24.0(6.1)								+			+	+
19	24.0 (6.3)	+	+	+	+			+	+		+	+	+

Spot No.	Mr – pI	T ₃	T ₆	T ₃	T ₆	T ₃	T ₆	T ₃	T ₆	T ₃	T ₆	T ₃	T ₆
20	24.2 (6.1)								+			+	+
21	24.7 (6.2)								+				+
22	24.9 (6.1)		+						+				+
23	25.6 (6.6)		+		+								
24	25.6 (6.8)				+		+				+		
25	25.8 (5.6)						+	+	+		+		
26	25.8 (6.5)				+	+	+	+	+		+		
27	26.5 (6.3)				+		+						
28	26.5 (6.8)	+	+		+		+				+		+
29	26.6 (6.3)		+		+	+	+						+
30	27.0 (6.2)		+			+	+			+	+		+
31	27.0 (6.5)	+	+		+								
32	27.6 (6.8)						+		+		+		
33	27.7 (6.3)						+				+		
34	27.7 (7.0)					+	+				+		
35	28.8 (6.5)						+		+		+		+
36	28.8 (6.8)						+		+		+		
37	29.0 (6.3)	+	+						+		+		+
38	29.0 (6.6)						+		+		+		
39	29.0 (8.3)		+		+								
40	29.4 (6.1)	+	+								+		
41	29.4 (6.7)						+		+		+		
42	30.4 (6.3)								+				
43	30.7 (8.3)		+		+				+				

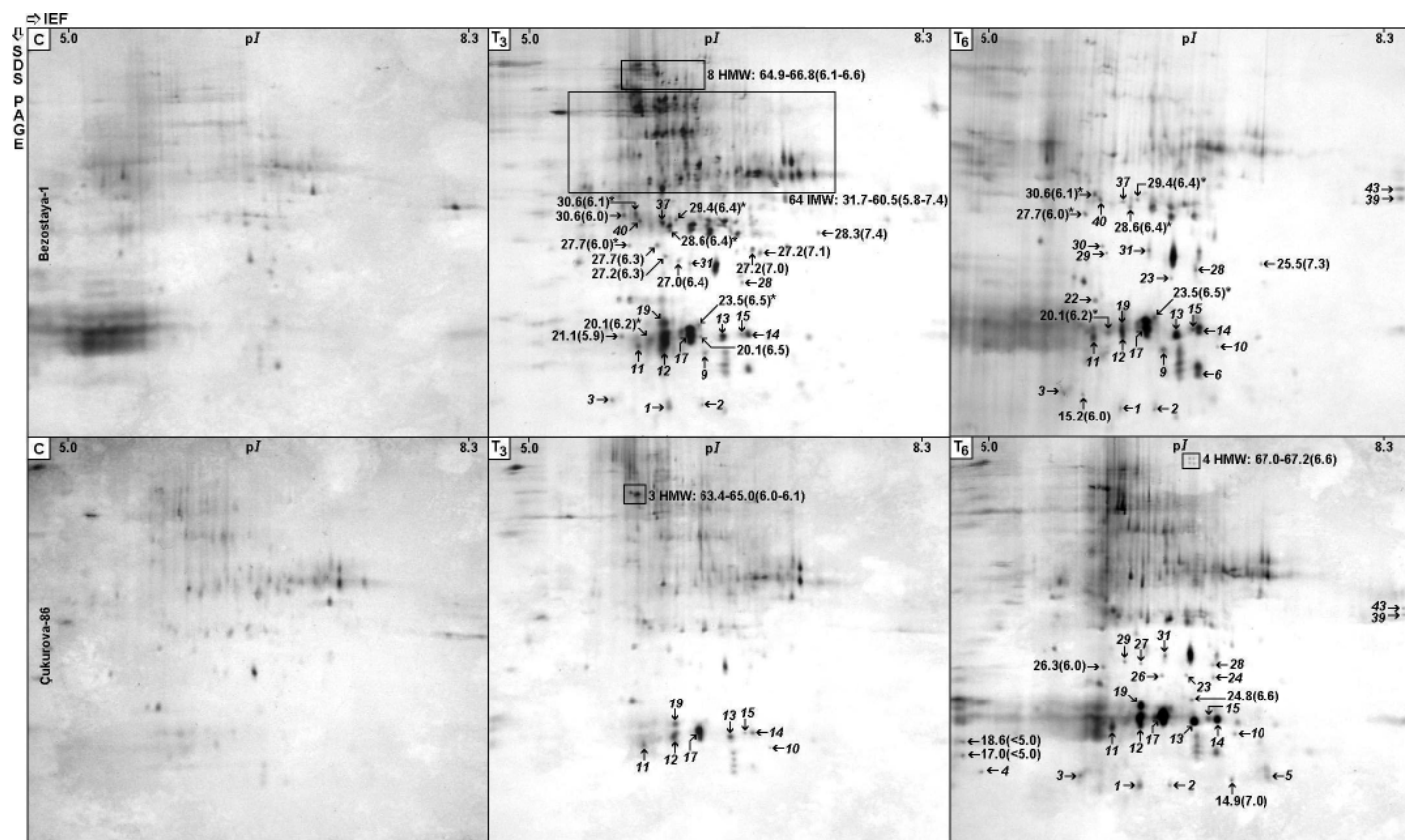


Fig. 4a. 2-D patterns of heat shock proteins (HSPs) extracted from seminal root samples of wheat cultivars (Bezostaya-1 and Cukurova-86) at control (C = 23 °C, 24 h), acclimation (T₃ = 37 °C, 24 h), and heat shock after acclimation (T₆ = 37 °C, 24 h → 50 °C, 1 h) temperature treatments. The HSPs are indicated by arrows. The numbers (1–43) indicate the low molecular weight (LMW) HSPs synthesized between at least two genotypes (see Table 1). 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 T₃ and T₆ temperature treatments within each genotype. The intermediate (IMW) and high molecular weight (HMW) HSPs are shown in boxes

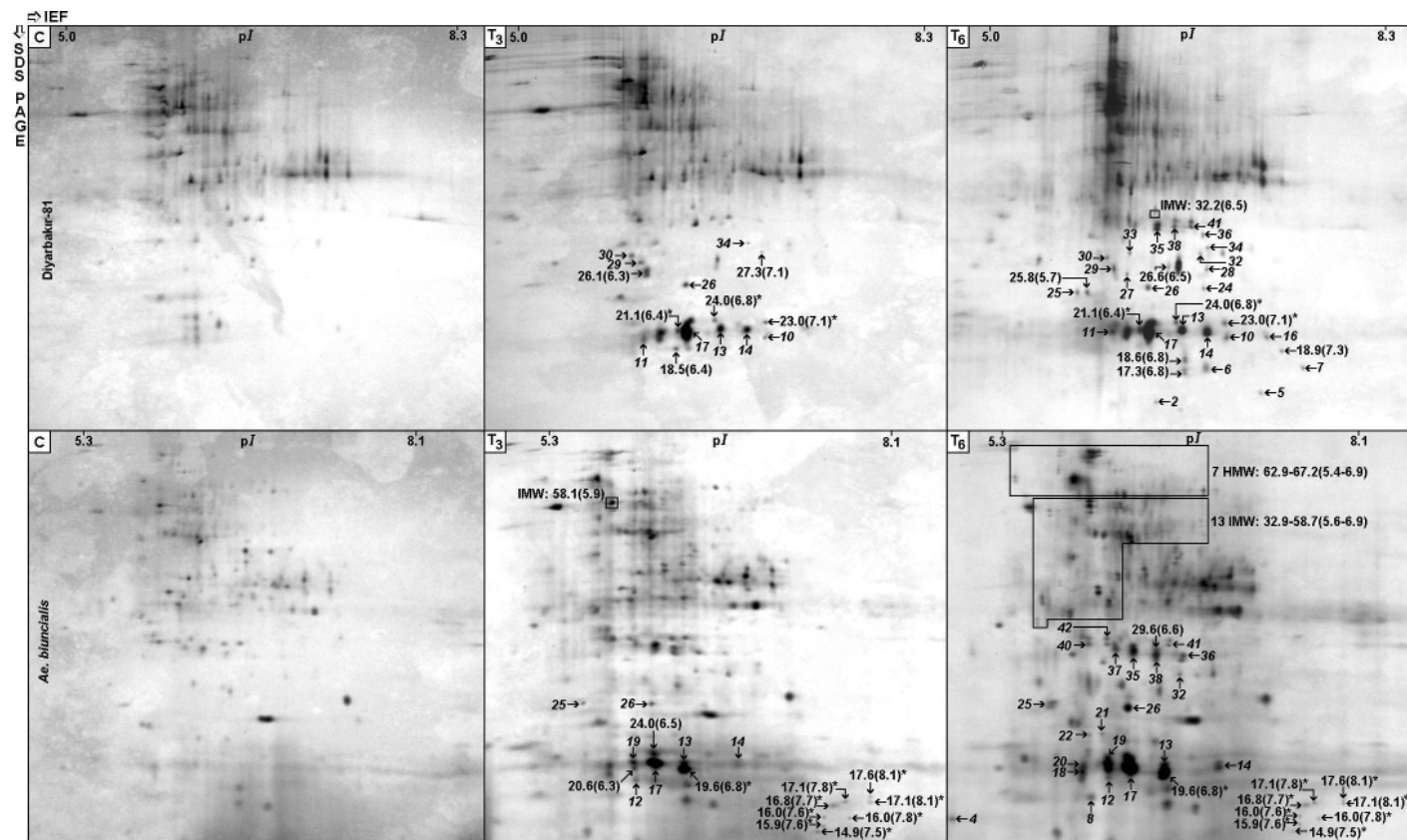


Fig. 4b. 2-D patterns of heat shock proteins (HSPs) extracted from seminal root samples of wheat cultivar (Diyarbakir-81) and wild wheat species (*Ae. biuncialis*) at control (C = 23 °C, 24 h), acclimation (T₃ = 37 °C, 24 h), and heat shock after acclimation (T₆ = 37 °C, 24 h → 50 °C, 1 h) temperature treatments. The HSPs are indicated by arrows. The numbers (1–43) indicate the low molecular weight (LMW) HSPs synthesized between at least two genotypes (see Table 1). 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 T₃ and T₆ temperature treatments within each genotype. The intermediate (IMW) and high molecular weight (HMW) HSPs are shown in boxes

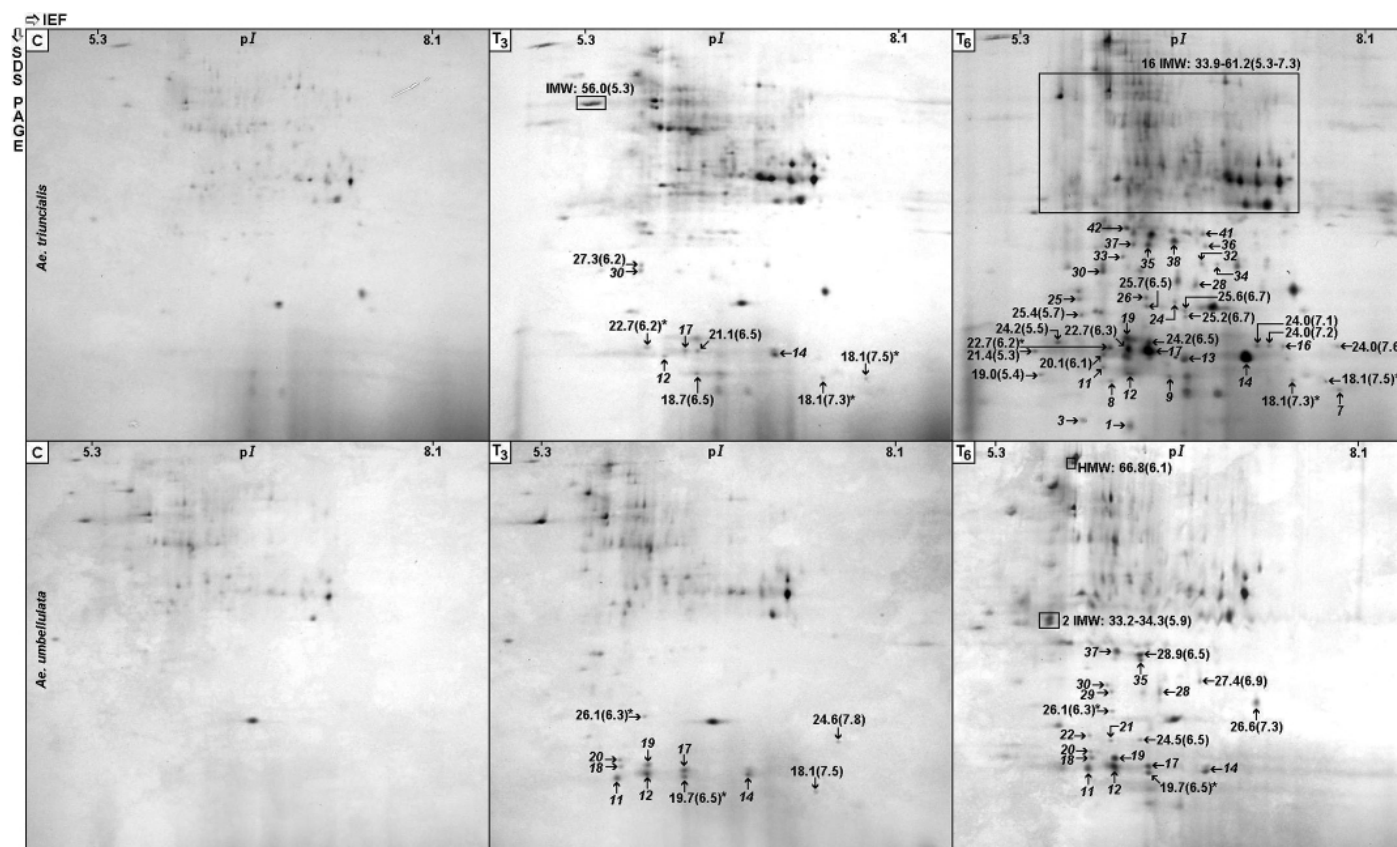


Fig. 4c. 2-D patterns of heat shock proteins (HSPs) extracted from seminal root samples of wild wheat species (*Ae. triuncialis* and *Ae. umbellulata*) at control (C = 23 °C, 24 h), acclimation (T₃ = 37 °C, 24 h), and heat shock after acclimation (T₆ = 37 °C, 24 h → 50 °C, 1 h) temperature treatments. The HSPs are indicated by arrows. The numbers (1–43) indicate the low molecular weight (LMW) HSPs synthesized between at least two genotypes (see Table 1). 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 T₃ and T₆ temperature treatments within each genotype. The intermediate (IMW) and high molecular weight (HMW) HSPs are shown in boxes

thesized in higher plants were more and different than in other eukaryotics. The most common LMW HSPs (1–43 protein spots) were ranged between 14.2 and 30.7 kDa (pI <5.0–8.3). These proteins were synthesized between at least two genotypes (Table 1 and Fig. 4a–c). The 20.1 (pI 7.0) and 22.1 kDa (pI 6.5) LMW HSPs were synthesized in all the genotypes (Table 1 and Fig. 4a–c). Some HSPs (with and without asterisk) were specific to genotype (Fig. 4a–c): That is, cv. B-1, cv. Ç-86, cv. D-81, *Ae. bi*, *Ae. tri* and *Ae. umb* showed 2 unique LMW HSP spots in the 15.2–25.5 kDa (pI 6.0–7.3), 5 in the 14.9–26.3 kDa (pI <5.0–7.0) range, 5 in the 17.3–26.6 kDa (pI 5.7–7.3), one in the 29.6 kDa (pI 6.6), 13 in the 19.0–25.7 kDa (pI 5.3–7.6) and 4 in the 24.5–28.9 kDa (pI 6.5–7.3), respectively, at T_6 temperature treatment. The 33.9 (pI 5.6), 34.3 (pI 5.9) and 36.4 kDa (pI 5.7) IMW HSPs were not determined in cultivated wheat cultivars while they were determined in *Ae. bi* and *Ae. tri* (Fig. 4a–c). Also, the 34.3 kDa (pI 5.9) HSP was determined in *Ae. umb* (Fig. 4c). The number of the HMW HSPs synthesized in cv. Ç-86, *Ae. bi* and *Ae. tri* are 4, 7 and 1 at T_6 temperature treatment, respectively. These proteins ranged between 66.8–67.2 kDa (pI 5.8–6.0) (Fig. 4a–c). Genetic variability in HSPs have been identified in root tissues of maize [5, 6], and durum and bread wheat, barley and rye [21]. In our research, HSPs showed genetic variability in *Triticum* cultivars and *Aegilops* species which are genetically related.

Some researchers reported that HSPs plays a role in cell protection, survival, and recovery in several species [22, 30]. HSPs are chaperons, which function during normal cell growth and stress conditions [19]. In our study, seedlings survived at 50 °C for 1 h because the seminal roots gained thermal tolerance at 37 °C for 24 h treatment. The HSPs may play an important role in protection of more sensitive roots and contribute to regeneration in partially injured roots. As a result of these, a vital relation may be assumed in acquiring thermal tolerance between coleoptile and root. These proteins may function in the acquisition of thermal tolerance. Also, Krishnan et al. [14] reported that LMW HSPs have an adaptive role at thermal tolerance. Some researchers documented that there was a positive correlation between the synthesis of HSPs and the acquisition of thermal tolerance [3, 17, 26, 28, 29].

In our research, synthesis of HSPs in seminal root tissues of genotypes was realized in early and late synthesis phases. That is, early HSPs were synthesized only at acclimation temperature (T_3), while late HSPs were synthesized only at acclimation temperature followed by heat shock (T_6). Necchi et al. [21] reported that gradual synthesis of new HSPs takes place in the root tissues of some monocotyledons at 40 °C for 4, 7 and 12 h.

In summary, the acclimation seedlings exhibited higher recovery growth, which was associated with higher expression of HSPs. The genetic variability for thermal tolerance was observed in seedlings as well as plants after subjecting them to acclimation temperatures. The variation in the thermal tolerance among genotypes has been predicted based on differential levels of basal as well as induced expression of HSPs.

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