Pak. J. Bot., 40(1): 317-327, 2008.

EVALUATION OF ACQUIRED THERMOTOLERANCE IN WHEAT (*TRITICUM AESTIVUM* AND *T. DURUM*) CULTIVARS GROWN IN TURKEY

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Abstract

Genotypic variability in acquired thermotolerance (ATT) among 30 cultivars of bread (*Triticum aestivum* L.) and durum (*Triticum durum* Desf.) wheat was evaluated at the seedling stage of growth by 2,3,5-triphenyltetrazolium chloride (TTC) cell viability and chlorophyll (Chl *a*+*b*) accumulation assays. In TTC assay, first leaf segments were subjected to heat stress (50°C, 1 h) after acclimation (37°C, 24 h) of 5-day old seedlings. In Chl accumulation assay, 5-day old etiolated seedlings were exposed to 25°C (24 h), 37°C (24 h) or 37°C (24 h)→50°C (1 h) treatments in dark. Etiolated seedlings were returned to optimum temperature at continuous light for Chl accumulation. Genotypic differences existed among cultivars tested for TTC at the seedling growth stage. Average acquired thermotolerance (ATT) value of all cultivars was 30.86%. Heat stress applied before greening of etiolated seedlings decreased Chl accumulation. High temperature treatments caused generally less injury to Chl pigmentation of bread wheat cultivars compared to durum wheat cultivars. Based on Chl accumulation, average ATT of all cultivars was 48.40% in 37°C→50°C/25°C ratio. Compared to Chl, carotenoid accumulation was less sensitive to direct high temperature treatment (50°C, 1 h) after acclimation treatment. The decrease in chlorophyll/carotenoid ratio of bread wheat cultivars was lower than that of durum wheat cultivars. Following the post-heat stress, the carotenoid content of bread wheat cultivars was lower than that of durum wheat cultivars and thus the decrease in chlorophyll/carotenoid ratio was found at low level. TTC and Chl accumulation tests were found to be appropriate for monitoring high temperature stress.

Introduction

High temperature limits productivity in several important crops (Fokar *et al*., 1998). Most of the world crops are exposed to heat stress during some stages of their life cycle (Stone, 2001; Sethar *et al.,* 2002). Severe heat stress (lethal dose) leads to cellular damage and cell death, sublethal doses of heat stress induce a cellular response, the heat shock response, which (a) protects cells and organisms from severe damage, (b) allows resumption of normal cellular and physiological activities, and (c) leads to a higher level of thermotolerance (Schöffl *et al*., 1998). Heat is a complex stress causing damage to a range of cellular components, so it should not be surprising that a large number of different protective pathways are required in order to survive. Induction of any one of these pathways allows the plant to acquire some measure of thermotolerance, and the loss of any specific pathway merely limits the extent of that tolerance (Larkindale *et al*., 2005). For cultivation in warmer periods or regions, it is essential to understand the seedling response to elevated temperature prior to assessment of wheat cultivars for thermotolerance (Porter & Gawith, 1999). Acquired thermotolerance means the level of protection beyond the inherent thermotolerance that the results from prior exposure to

elevated, non-lethal temperatures (Burke, 1998). In plant cells, membrane function is especially important for membrane-based processes such as photosynthesis and respiration. Cellular regrowth, electrolyte leakage, 2,3,5-triphenyltetrazolium chloride (TTC) reduction, and chlorophyll accumulation assays have been used for identifying genetic variability in acquired thermotolerance (Wu & Wallner, 1983; Sethar *et al.,* 1997; Dash & Mohanty, 2001; Ibrahim & Quick, 2001; Camejo *et al*., 2005). One of commonly used assays for heat tolerance in plants is cell viability assay based on TTC reduction which is related to mitochondrial membranes (Blum, 1988). The dehydrogenase systems are responsible for TTC reduction (Roberts, 1951). TTC reduction has been widely used in the viability assay of plant tissues exposed to high temperature, and genotypic differences in thermotolerance were evaluated in different plant tissues (Chen *et al*., 1982; Wu & Wallner, 1983; Krishnan *et al*., 1989; Porter *et al*., 1994; Fokar *et al*., 1998; Mullarkey & Jones, 2000; Ibrahim & Quick, 2001; Dhanda & Munjal, 2006; Yıldız & Terzioğlu, 2006). The chlorophyll bioassay for high temperature induced injury has been used in the evaluation of acquired thermotolerance in many crop species and *Arabidopsis thaliana* (Burke, 1994; 1998; Burke *et al*., 2000; O'Mahony *et al*., 2000; Dash & Mohanty, 2001; Camejo *et al*., 2005).

The aim of this research was to evaluate the genotypic variability in acquired thermotolerance in seedlings of 16 bread wheat (*Triticum aestivum* L.) and 14 durum wheat (*Triticum durum* Desf.) cultivars using TTC reduction cell viability and chlorophyll accumulation assays following different temperature treatments.

Materials and Methods

Plant material and growth conditions: Sixteen bread wheat (*Triticum aestivum* L.) and 14 durum wheat (*Triticum durum* Desf.) cultivars were evaluated for acquired thermotolerance as measured by TTC reduction and Chl accumulation assays controlled environment experiments. Seeds were obtained from Agricultural Research Institutes in different regions of Turkey. Seeds were germinated on moistened, folded germination paper at 25°C. Two-day old etiolated seedlings were grown in a controlled growth chamber with a 16 h photoperiod, a light intensity of 110 μmol photons m^{-2} s^{-1} (Sylvania Gro-Lux fluorescent lamp, F18W/GRO) and 60% relative humidity during 3 days for TTC reduction assay. In Chl accumulation assay, however, the etiolated seedlings were grown in dark during 3 days. After heat treatments, the 6-d old etiolated seedlings were subjected to the continuous light for 24 h.

Cell viability (TTC reduction assay): Five-day old seedlings were acclimated at 37°C for 24 h. Following acclimation, immediately four leaf segments (2 cm each) were excised from two first leaves after removing 1 cm segment tip side. For high temperature treatment, four leaf segments were placed in a test tube with 100 μL distilled water and test tubes transferred in a water bath at 50ºC for 1 h. After acclimation and high temperature treatments, 4 mL of TTC solution (8 mg mL^{-1} TTC in 0.05 M K_2PO_4 buffer, pH 7.5, and 0.5 mL L^{-1} Tween 80) was immediately added to each tube, and infiltrated for approximately 15 min., by vacuum using the procedure of Porter *et al*., (1994) with minor modification. Then, leaf segments were removed and rinsed three times with distilled water. The formazan, which was produced by TTC reduction, was extracted with 950 mL L^{-1} ethanol at 25ºC for 24 h in the dark. The level of formazan was assayed by reading the optical density (OD) at 530 nm with a double beam spectrophotometer

(TU-1880 Double Beam UV-VIS). The level of acquired thermotolerance (ATT) was determined by measuring the percentage reduction of TTC to formazan using the following formula:

$$\text{Acquired thermotolerance (\%)} = (OD_{37^oC \rightarrow 50^oC} / OD_{37^oC}) \times 100$$

Extraction and estimation of photosynthetic pigments: Five-day old etiolated seedlings were separated into three sets per cultivar. The seedlings were exposed to the following treatments:

Control treatment

25°C (120 h) $_{\text{Dark}} \rightarrow$ 25°C (24 h) $_{\text{Dark}} \rightarrow$ 25°C (24 h) $_{\text{Continuous light}}$

Acclimation (sublethal) treatment

25°C (120 h) $_{\text{Dark}} \rightarrow$ 37°C (24 h) $_{\text{Dark}} \rightarrow$ 25°C (24 h) $_{\text{Light}}$

Acclimation $\rightarrow$ high temperature (letal) treatment

25°C (120 h) $_{\text{Dark}} \rightarrow$ 37°C (24 h) $_{\text{Dark}} \rightarrow$ 50°C (1 h) $_{\text{Dark}} \rightarrow$ 25°C (24 h) $_{\text{Continuous light}}$

The seedlings subjected to control, acclimation, and acclimation$\rightarrow$high temperature treatments were re-grown at 25°C for 24 h post heat-stress growth.

After all the treatments, total leaf chlorophylls (Chl *a*+*b*) and carotenoids were extracted and estimated according to Wellburn (1994). About 45 mg of first leaves were cut into tiny segments and kept in 10 mL of chilled absolute methanol in a capped glass tube. After 48 h extraction in dark at 4ºC and thus, the leaf segments were well-extracted for residual pigments. The concentration of total Chl and carotenoids was measured from optical density (OD) readings of extracts at 666, 653 and 470 nm using a double beam spectrophotometer (TU-1880 Double Beam UV-VIS), and calculated as per the equation of Wellburn (1994):

$$\text{Chl } a = 15.65A_{666} - 7.34A_{653} \times D^*$$
$$\text{Chl } b = 27.05A_{653} - 11.21A_{666} \times D$$
$$\text{Carotenoids} = (1000A_{470} - 2.86\text{Chl } a - 129.2\text{Chl } b) / 221 \times D$$

***** D = Dilution coefficient

At 37°C and 37$\rightarrow$50°C temperature treatments compared to control, the decrease of total Chl accumulation was calculated using following formulas:

$$\%\ \text{Decrease}_{(\text{Chl } a+b \text{ accumulation})} = [1 - (\text{Chl } a+b_{37^oC} / \text{Chl } a+b_{25^oC})] \times 100$$
$$\%\ \text{Decrease}_{(\text{Chl } a+b \text{ accumulation})} = [1 - (\text{Chl } a+b_{37\rightarrow 50^oC} / \text{Chl } a+b_{25^oC})] \times 100$$

At 37°C and 37$\rightarrow$50°C temperature treatments compared to control, the decrease of Chl *a*+*b*/Carotenoid (Chl *a*+*b*/Carot) accumulation was calculated using following formulas:

$$\%\ \text{Decrease}_{(\text{Chl } a+b/\text{Carot})} = 1 - [(\text{Chl } a+b/\text{Carot})_{37^oC} / (\text{Chl } a+b/\text{Carot})_{25^oC}] \times 100$$
$$\%\ \text{Decrease}_{(\text{Chl } a+b/\text{Carot})} = 1 - [(\text{Chl } a+b/\text{Carot})_{37\rightarrow 50^oC} / (\text{Chl } a+b/\text{Carot})_{25^oC}] \times 100$$

Statistical analysis: The data were analyzed as a completely randomized design with 6 replicates using SPSS computer package for all sets of data, and means were compared using Duncan's multiple comparison and Student's-T tests at $p<0.05$ level.

Results and Discussion

Heat tolerance or acquired thermotolerance is quantified by mitochondrial reduction of TTC. The relative level of TTC reduction to formazan then quantifies cell viability by spectrophotometric assay of the red formazan (Towill & Mazur, 1975). In the present study, large and significant differences existed among cultivars tested for TTC at the seedling growth stage (Table 1). TTC reduction was decreased significantly at acclimation→high temperature (37°C, 24 h→50°C, 1 h) treatment compared to acclimation (37°C, 24 h) ($p<0.05$). Although root tissues of acclimated seedlings were injured partly from high temperature, their viability continued. The decrease in cell viability resulting from high temperature treatment may be attributed to uncoupling of the electron transport chain through disruption of the inner mitochondrial membrane and/or inactivation of enzymes of the respiratory pathway (Porter *et al*., 1994). Mean optical density (OD) values ranged from 1.262 (low) in durum wheat cv. Yelken-2000 to 4.607 (high) in bread wheat cv. İkizce-96 at acclimation treatment (Table 1). In addition, the OD values in the tissues ranged from 0.122 (low) in bread wheat cv. Sönmez-2001 to 2.658 (high) in bread wheat cv. İkizce-96 at acclimation→high temperature treatment. When these data were entered into the formula used to express the capacity of the leaf tissue to acquire thermotolerance (ATT) for each cultivar, the level of diversity of the response was dramatic. The values for acquired thermotolerance ranged from a high of 76.33% for bread wheat cv. Basribey-95 to a low of 7.91% for bread wheat cv. Sönmez-2001. ATT values of durum wheat cultivars ranged from 49.56% (high) in cv. Ankara-98 to 16.65% (low) in cv. Kunduru-1149. These results demonstrated that acclimated tissues were capable of acquiring thermotolerance. ATT values between or among some cultivars were at same significant level ($p<0.05$). Average ATT value for all cultivars was calculated as 30.86% at the seedling stage. Based on average ATT value, thermal tolerance values of 7 bread and 5 durum wheat cultivars were above the ATT average while others were below the average. Similar results were reported by Ibrahim & Quick (2001) in winter and spring wheat. Analysis of variance showed highly significant variation among the 14 winter and spring wheat cultivars for TTC reduction. Based on average ATT value (58.5%), thermal tolerance levels of cultivars were between 80.8-82.2% (high), 54.7-63.9% (intermediate) and 20.4-39.8% (low) at same significant level, respectively (Ibrahim & Quick, 2001).

Fokar *et al*., (1998) reported that the results obtained by TTC did not change with plant age. However, differences in ATT values were determined when a cultivar was subjected to same temperature and period treatments at the different stages of seedling growth. In our study, ATT value in first leaf tissue of bread wheat cv. Bezostaya-1 was detected as 54.90% (Table 1). However, this value in coleoptile tissue of the same cultivar was detected as 37.82% (Yıldız & Terzioğlu, 2006). These results showed that coleoptile tissue was most sensitive to high temperature than the first leaf tissue. However, it should be noted that several studies have found differences in ATT values when a cultivar was subjected to different temperature and period treatments at the seedling stage (Porter *et al*., 1994; Fokar *et al*., 1998; Ibrahim & Quick, 2001). ATT value of Kauz wheat cultivar which exposed to 49°C (30 min) following 39°C (48 h) was 82.2% (Ibrahim & Quick, 2001) while this value was 18.7% when the seedlings were subjected to 50°C (1 h) following 34°C (24 h). In addition, ATT values differed among

Table 1. Acquired thermotolerance (ATT %) as estimated by cell viability (TTC) assay in 16 bread and 14 durum cultivars of wheat at the seedling stage.

Cultivars	Temperature treatment		ATT (%)
	37°C	37°C→50°C	
	OD_{530}		
Basribey-95	1.467 ± 0.064 **A***	1.119 ± 0.048 **B***	76.33 ± 3.30 **a****
Amanos-97***	2.458 ± 0.128 **A**	1.844 ± 0.048 **B**	75.01 ± 1.94 **a**
Gerek-79	3.519 ± 0.090 **A**	2.208 ± 0.067 **B**	62.74 ± 1.91 **b**
İkizce-96	4.607 ± 0.066 **A**	2.658 ± 0.060 **B**	57.70 ± 1.31 **c**
Bezostaya-1	1.729 ± 0.053 **A**	0.949 ± 0.032 **B**	54.90 ± 1.85 **cd**
Ceyhan-99	1.887 ± 0.078 **A**	0.992 ± 0.020 **B**	52.56 ± 1.04 **de**
Ankara-98	2.436 ± 0.118 **A**	1.207 ± 0.056 **B**	49.56 ± 2.32 **ef**
Ege-88	2.432 ± 0.023 **A**	1.184 ± 0.030 **B**	48.70 ± 1.24 **f**
Fuatbey-2000	2.724 ± 0.021 **A**	0.862 ± 0.040 **B**	31.64 ± 1.47 **g**
Gönen-98	2.483 ± 0.037 **A**	0.773 ± 0.017 **B**	31.14 ± 0.70 **g**
Çakmak-79	1.329 ± 0.019 **A**	0.404 ± 0.014 **B**	30.40 ± 1.06 **g**
Gün-91	2.234 ± 0.045 **A**	0.679 ± 0.007 **B**	30.37 ± 0.33 **g**
İzmir-85	2.581 ± 0.047 **A**	0.661 ± 0.023 **B**	25.60 ± 0.90 **h**
Yelken-2000	1.262 ± 0.043 **A**	0.314 ± 0.006 **B**	24.84 ± 0.44 **h**
Gediz-75	2.653 ± 0.050 **A**	0.636 ± 0.014 **B**	23.98 ± 0.52 **hi**
Kızıltan-91	1.709 ± 0.031 **A**	0.405 ± 0.028 **B**	23.69 ± 1.63 **hij**
Aydın-93	1.732 ± 0.071 **A**	0.402 ± 0.031 **B**	23.23 ± 1.84 **hij**
Adana-99	1.681 ± 0.096 **A**	0.382 ± 0.025 **B**	22.72 ± 1.47 **hij**
Salihli-92	2.339 ± 0.059 **A**	0.478 ± 0.014 **B**	20.43 ± 0.60 **ijk**
Tüten-2002	1.937 ± 0.062 **A**	0.383 ± 0.027 **B**	19.75 ± 1.42 **jkl**
Şölen-2002	1.772 ± 0.054 **A**	0.319 ± 0.007 **B**	18.02 ± 0.41 **klm**
Çeşit-1252	2.711 ± 0.099 **A**	0.486 ± 0.024 **B**	17.92 ± 0.87 **klm**
Kunduru-1149	1.767 ± 0.052 **A**	0.294 ± 0.014 **B**	16.65 ± 0.82 **klm**
Meta-2002	3.165 ± 0.092 **A**	0.518 ± 0.033 **B**	16.36 ± 1.05 **klm**
Kaşifbey-95	3.543 ± 0.047 **A**	0.554 ± 0.017 **B**	15.63 ± 0.48 **lm**
Cumhuriyet-75	2.193 ± 0.057 **A**	0.337 ± 0.012 **B**	15.38 ± 0.56 **m**
Pandas	2.825 ± 0.099 **A**	0.408 ± 0.020 **B**	14.42 ± 0.70 **m**
Altay-2000	3.911 ± 0.046 **A**	0.393 ± 0.022 **B**	10.05 ± 0.57 **n**
Ziyabey-98	2.945 ± 0.100 **A**	0.244 ± 0.011 **B**	8.27 ± 0.39 **n**
Sönmez-2001	1.540 ± 0.020 **A**	0.122 ± 0.006 **B**	7.91 ± 0.38 **n**
		Average	**30.86 ± 1.44**

*Mean values and standard error (±SE) followed by the capital letters in rows are not significantly different according to the Student's t-test.

**Mean values and standard error (±SE) followed by the same letters in ATT column are not significantly different according to the Duncan's multiple range test.

***Durum wheat cultivars are shown as bold.

some wheat cultivars (Seri 82, V5, Siete Cerros and Deberia) which were tested in these studies. On the other hand, differences in ATT values of 8 cultivars of spring wheat were determined between the seedling and the flowering growth stages (Fokar *et al*., 1998). These researchers found that ATT in the seedling stage differed than that of the flowering

stage. But average ATT value over all cultivars did not differ significantly between the seedling and the flowering growth stages (Fokar *et al*., 1998). In the present study, TTC reduction test was used to measure cultivar differences in acquired thermal tolerance at the seedling stage of bread and durum wheat cultivars. However, to establish the relationship (plant's capacity to acquire thermal tolerance) among the seedling, flowering and grain-filling stages is important for estimation of the level of acquired thermal tolerance.

Plant re-growth, electrolyte leakage and TTC reduction are commonly used procedures for evaluating thermotolerance (Wu & Wallner, 1983). Burke (1994), however, has developed a simple, species-non-specific, reliable, and accurate protocol for the quantification of thermotolerance. This protocol is based on the inhibition of chlorophyll accumulation in etiolated tissue by challenges at lethal temperatures and the prevention of this inhibition by pre-incubation at a non-lethal elevated temperature; i.e. acquired thermotolerance. Changes in the temperature sensitivity of chlorophyll accumulation were used as an indicator of acquired thermal tolerance (Burke & Oliver, 1993; Burke, 1998; Burke *et al*., 2000; O'Mahony *et al*., 2000; Camejo *et al*., 2005). Plants and other organisms have both an inherent ability to survive exposure to temperatures above the optimal for growth (basal thermotolerance, BTT) and an ability to acquire tolerance to otherwise lethal heat stress (acquired thermotolerance, ATT). Acquired thermotolerance is induced by a short acclimation period at moderately high (but survivable) temperatures or by treatment with other non-lethal stress prior to heat stress (Burke *et al*., 2000). In the light of this knowledge, total chlorophyll (Chl *a*+*b*) accumulation in 37°C/25°C and 37°C→50°C/25°C ratios may be evaluated as the BTT and the ATT, respectively. In this sense, the BTT of bread wheat cv. Ceyhan-99 (15.37% inhibition) was highest among all cultivars while the BTT of durum wheat cv. Kızıltan-96 (61.97% Chl inhibition) was lowest (Table 2). However, the ATT of bread wheat cv. Gönen-98 (32.13% Chl inhibition) was highest among all cultivars while the ATT of durum wheat cv. Ankara-98 (67.14%) was lowest. Based on BTT and ATT averages (34.22% and 48.40%, respectively), the cultivars determined at below and above of averages were almost same (Table 2). In addition, high temperature treatments caused generally less injury to chlorophyll pigmentation of bread wheat cultivars (ABD genome) compared to durum wheat cultivars (AB genome). O'Mahony *et al*., (2000) reported that ditelosomic line (DT7DS line which has no short arm of 7D chromosome) could only accumulate approximately 7% of the chlorophyll that the wild-type line accumulated at heat stress. Thus it appears that the DT7DS line is not capable of establishing a significant level of acquired thermotolerance following a 40°C for 4 h pre-incubation that would protect the metabolic activities associated with chlorophyll accumulation in the light from a high temperature challenge.

Since heat-stress to wheat seedlings induces aging of first leaves (Mohanty *et al*., 1987), the period of post-stress growth was limited to 24 h in order to avoid undesirable effects of senescence on recovery processes (Dash & Mohanty, 2001). However, optimal rates of photosynthesis in wheat leaves are broader, with an optimum temperature of 25°C (Porter & Gawith, 1999). In the present study, therefore, optimum temperature for chlorophyll accumulation of etiolated wheat seedlings was 25°C and exposure time to continuous light at 110 μmol m^{-2} s^{-1} was limited to 24 h. Heat stress treated before greening of etiolated seedlings decreased chlorophyll accumulation. Following post-stress growth at 25°C after 25°C, 37°C, and 37°C→50°C treatments, the foliar levels of Chl *a*+*b* in all

Table 2. Effect of the high temperature treatments on total Chl a+b accumulation in first leaf tissues of bread and durum wheat at the seedling stage (%).

Cultivars	Chl *a*+*b* accumulation % Decrease (37ºC/25ºC)	Cultivars	Chl *a*+*b* accumulation % Decrease (37→50ºC/25ºC)
Ceyhan-99	15.37 ± 2.58 **a***	Gönen-98	32.13 ± 2.14 **a**
Bezostaya-1	15.38 ± 1.47 **a**	Pandas	33.62 ± 0.98 **a**
Adana-99	19.60 ± 2.10 **ab**	Ziyabey-98	34.08 ± 2.02 **a**
Basribey-95	21.05 ± 3.08 **ab**	Bezostaya-1	34.96 ± 1.67 **ab**
Sönmez-2001	21.38 ± 2.72 **ab**	Kaşifbey-95	35.59 ± 2.21 **ab**
Gediz-75**	22.33 ± 1.06 **b**	Ceyhan-99	36.05 ± 2.11 **ab**
Gönen-98	22.43 ± 1.51 **b**	İzmir-85	37.30 ± 2.05 **ab**
Kaşifbey-95	23.18 ± 1.70 **b**	Gün-91	40.21 ± 2.11 **bc**
İzmir-85	23.50 ± 2.46 **b**	Meta-2002	40.28 ± 2.50 **bc**
Pandas	23.70 ± 2.50 **b**	Sönmez-2001	40.46 ± 1.76 **bc**
Ziyabey-98	23.98 ± 2.80 **b**	Gerek-79	42.84 ± 2.52 **c**
Gerek-79	24.25 ± 2.66 **b**	Adana-99	44.82 ± 2.14 **cd**
Ege-88	30.32 ± 1.37 **c**	**Ege-88**	45.19 ± 1.34 **cd**
Altay-2000	31.09 ± 2.26 **c**	**Gediz-75**	45.78 ± 0.84 **cd**
Gün-91	32.84 ± 2.03 **c**	Basribey-95	48.68 ± 2.25 **de**
Meta-2002	34.14 ± 2.29 **cd**	Altay-2000	51.00 ± 0.97 **ef**
Amanos-97	35.96 ± 1.25 **cde**	**Çeşit-1252**	52.38 ± 1.56 **efg**
Aydın-93	36.29 ± 2.64 **cde**	**Amanos-97**	52.94 ± 1.59 **efg**
Salihli-92	39.58 ± 0.94 **def**	**Çakmak-79**	52.95 ± 1.26 **efg**
Cumhuriyet-75	39.67 ± 0.65 **def**	**Salihli-92**	54.27 ± 1.42 **fg**
Çakmak-79	41.02 ± 1.00 **efg**	İkizce-96	54.53 ± 1.62 **fg**
Fuatbey-2000	41.93 ± 1.40 **efg**	**Fuatbey-2000**	55.02 ± 2.22 **fgh**
Şölen-2002	42.92 ± 1.68 **fg**	**Tüten-2002**	56.74 ± 2.15 **gh**
Çeşit-1252	44.62 ± 2.62 **fgh**	**Aydın-93**	57.35 ± 2.14 **ghij**
Tüten-2002	46.70 ± 1.70 **ghi**	Cumhuriyet-75	60.06 ± 1.41 **hij**
Yelken-2000	49.35 ± 2.25 **hij**	**Yelken-2000**	60.58 ± 1.31 **ij**
İkizce-96	50.73 ± 1.19 **ijk**	**Kunduru-1149**	60.94 ± 1.43 **ij**
Kunduru-1149	55.16 ± 0.72 **jk**	**Şölen-2002**	61.71 ± 1.15 **ij**
Ankara-98	56.26 ± 1.88 **k**	**Kızıltan-91**	62.55 ± 1.01 **jk**
Kızıltan-91	61.97 ± 2.41 **l**	**Ankara-98**	67.14 ± 0.86 **k**
Average	**34.22 ± 1.01**	**Average**	**48.40 ± 0.81**

*Mean values and standard error (±SE) followed by the same letters in Chl *a*+*b* accumulation column are not significantly different according to the Duncan's multiple range test.
**Durum wheat cultivars are shown as bold.

cultivars ranged between 1.14-1.43 mg.g^{-1} fresh weight (FW), 0.51-1.15 mg.g^{-1} FW, and 0.42-0.86 mg.g^{-1} FW, respectively. Chlorophyll accumulation was decreased at high temperature treatments as compared to the control. It is suggested that this decrement might be caused from inhibition of the enzymes which play a role in Chl biosynthesis. Tewari & Tripathy (1998) reported that inhibition of Chl biosynthesis was partly due to impairment of 5-aminolevulinic acid biosynthesis in heat-stress conditions. In heat-stressed seedlings,

5-aminolevulinic acid dehydratase and porphobilinogen deaminase were partially inhibited (Tewari & Tripathy, 1998). On the other hand, the chlorophyll accumulation ($mg.g^{-1}$ FW) varied within a narrow range at 25°C while it varied within a wide range at heat stress treatments. In this sense, high temperature treatments are important for determining the genetic variability in thermal tolerance based on chlorophyll accumulation.

In 37°C/25°C ratio, the decrease in chlorophyll accumulation was below (15.37-49.35%) 50% in cultivars except for İkizce-96, Kunduru-1149, Ankara-98 and Kızıltan-91 cultivars (50.73-61.7%). However, in 37°C→50°C/25°C ratio, the decrease in chlorophyll accumulation ranged from 32.13% to 67.14%. O'Mahony *et al*., (2000) reported that inhibition of chlorophyll accumulation in Chinese spring wheat was 95% at 48°C/30°C. However, acclimation at 40°C prior to 48°C lethal temperatures caused 48% reduction in chlorophyll accumulation. Acclimation at 40°C delivered the maximum level of protection. In the present study, average Chl accumulation of all cultivars was 48.40% in 37°C→50°C/25°C ratio. Therefore, acclimation treatment provided an important protection to 50°C.

The carotenoid accumulation differed among wheat cultivars in 37°C/25°C and 37°C→50°C/25°C ratios. In these ratios (%), the inhibition of carotenoid accumulation in first leaf tissues of different cultivars was between 2.44-30.05% and 0.29-32.67%, respectively. Compared to chlorophyll, carotenoid accumulation was less sensitive to directly high temperature treatment (50°C, 1 h) after acclimation treatment. Similarly, Dash & Mohanty (2001) reported that the inhibition of carotenoid pigmentation of 8 bread wheat cultivars exposed to post heat-stress treatment was lower than that of chlorophyll pigmentation. In the present study, chlorophyll/carotenoid ratio decreased significantly with an increase in temperature. Average values of the chlorophyll/carotenoid ratio of all cultivars in 37°C/25°C and 37°C→50°C/25°C ratios were 24.03 and 43.31%, respectively (Table 3). Most of the bread wheat cultivars were above the mean values of chlorophyll/carotenoid ratio. The decrease in chlorophyll/carotenoid ratio of bread wheat cultivars was lower than durum wheat cultivars. Following the post-heat stress, the carotenoid content of bread wheat cultivars was lower than that of durum wheat cultivars and thus the decrease in chlorophyll/carotenoid ratio was found at low level. The etiolated seedlings were re-grown in continuous light at 25°C (optimum temperature) following heat stress treatments. We suggest that the mechanism of chlorophyll biosynthesis in the present optimum conditions, and therefore there is no more requirement to carotenoids which play the protective role in light-harvesting systems. Therefore, it is concluded that the bread wheat cultivars may be evaluated as more thermotolerant than the durum wheat cultivars. In contrast to this result, carotenoid amount in wild tomato genotype (thermotolerant cv. Nagcarlang) was increased while chlorophyll/carotenoid ratio was decreased. However, cultivated tomato (thermosensitive cv. Campbell-28) chlorophyll/carotenoid ratio was not changed (Camejo *et al*., 2005).

Development of heat-tolerant cultivars 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 (Fokar *et al*., 1998). Heat tolerance is not controlled by a single thermotolerant gene in cereals. Different components of tolerance determined by different sets of genes are critical for heat tolerance at different stages of the life cycle and in various tissues (Maestri *et al*., 2002). Hence, there is a strong need to elucidate molecular and genetic basis of heat tolerance in cereals, to identify beneficial genes and alleles, and to utilize them in the molecular breeding programs targeted to produce superior cereal cultivars in the future.

Table 3. Effect of the high temperature treatments on chlorophyll/carotenoid (Chl *a+b*/Carot) ratio in first leaf tissues of bread and durum wheat at the seedling stage (%).

Cultivars	Chl *a+b*/Carot % Decrease (37ºC/25ºC)	Cultivars	Chl *a+b*/Carot % Decrease (37→50ºC/25ºC)
Ceyhan-99	6.27 ± 0.10 **a***	Bezostaya-1	22.52 ± 3.98 **a**
Basribey-95	4.23 ± 2.25 **b**	Ceyhan-99	22.71 ± 3.45 **a**
Bezostaya-1	4.70 ± 2.80 **bc**	Gönen-98	26.49 ± 6.02 **ab**
Adana-99	9.33 ± 3.46 **bcd**	Basribey-95	29.03 ± 1.70 **abc**
Ziyabey-95	9.53 ± 7.66 **bcd**	Pandas	31.79 ± 2.50 **bcd**
Gediz-75**	12.24 ± 1.89 **bcd**	Adana-99	31.98 ± 2.53 **bcd**
Gerek-79	12.34 ± 1.48 **bcd**	İzmir-85	32.10 ± 1.60 **bcd**
Gönen-98	15.57 ± 3.55 **cde**	Gün-91	35.03 ± 2.48 **bcde**
Aydın-93	17.44 ± 3.64 **def**	Gerek-79	35.71 ± 3.50 **cdef**
Meta-2002	18.00 ± 5.46 **defg**	Kaşifbey-95	36.09 ± 1.78 **cdef**
Kaşifbey-95	19.28 ± 2.51 **defgh**	**Aydın-93**	36.29 ± 1.79 **cdef**
Gün-91	19.45 ± 1.39 **defgh**	Meta-2002	40.55 ± 4.43 **defg**
Ege-88	19.72 ± 2.38 **defgh**	Altay-2000	41.70 ± 1.03 **efgh**
Pandas	20.92 ± 1.83 **defgh**	Ziyabey-95	42.07 ± 5.54 **efgh**
Amanos-97	24.26 ± 2.75 **efghij**	**Amanos-97**	42.54 ± 1.82 **efgh**
Salihli-92	24.65 ± 0.73 **efghij**	Sönmez-2001	44.11 ± 2.80 **efghi**
Sönmez-2001	24.88 ± 4.61 **efghij**	İkizce-96	45.10 ± 3.78 **fghij**
İzmir-85	26.55 ± 2.65 **efghij**	**Ege-88**	46.41 ± 1.40 **ghijk**
Tüten-2002	28.25 ± 4.59 **fghij**	Cumhuriyet-75	47.10 ± 3.57 **ghijkl**
Cumhuriyet-75	28.82 ± 1.99 **fghij**	**Gediz-75**	47.75 ± 1.21 **ghijkl**
Altay-2000	29.27 ± 2.48 **ghij**	**Çakmak-79**	48.45 ± 3.58 **ghijkl**
Şölen-2002	30.25 ± 2.41 **hij**	**Çeşit-1252**	50.68 ± 2.05 **hijklm**
Fuatbey-2000	31.88 ± 2.39 **ijk**	**Salihli-92**	52.09 ± 2 .94 **ijklm**
İkizce-96	33.54 ± 3.24 **jk**	**Fuatbey-2000**	53.48 ± 2.12 **ijklm**
Çakmak-79	34.07 ± 2.53 **jk**	**Ankara-98**	54.20 ± 0.71 **jklm**
Çeşit-1252	41.30 ± 1.82 **kl**	**Kızıltan-91**	54.71 ± 2.14 **klm**
Ankara-98	44.91 ± 1.34 **l**	**Tüten-2002**	56.43 ± 2.06 **lmn**
Kızıltan-91	45.26 ± 2.21 **l**	**Kunduru-1149**	59.84 ± 1.34 **mn**
Yelken-2000	47.63 ± 2.03 **l**	**Yelken-2000**	63.77 ± 2.05 **no**
Kunduru-1149	48.99 ± 1.65 **l**	**Şölen-2002**	68.63 ± 1.86 **o**
Average	**24.03 ± 1.16**	**Average**	**43.31 ± 0.99**

*Mean values and standard error (±SE) followed by the same letters in Chl *a+b*/Carot column are not significantly different according to the Duncan's multiple range test.
**Durum wheat cultivars are shown as bold.

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(Received for publication 6 March 2007)