

PHYSIOLOGICAL EFFECTS OF HIGH TEMPERATURE TREATMENTS ON TOMATO LEAVES AT TWO DEVELOPMENTAL PHASES

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Abstract

This study was conducted to investigate the effects of high temperatures on three tomato cultivars at first bloom and yield stages. The leaves were subjected to high temperature stress at 35, 40, 45, 50, 55 and 60°C with gradual increments every 30-minutes in both stages. Samples were analyzed for total chlorophyll (Chl), carotenoid (Car), ascorbic acid (AsA), glutathione (GSH), total soluble protein (TSP) contents. Besides, protein profiles were determined with SDS-PAGE. Heat stress decreased Chl content in both stages, while it was higher in first bloom stage than in yield stage. Whereas carotenoid content increased in both stages. The AsA and GSH contents were higher in yield stage than in first bloom stage. Heat stress, generally reduced AsA content, while increased GSH content. It was observed that the effect of cultivars and temperature treatments on the TSP content was different in both periods. In addition, TSP content had decreased with increasing temperatures, while many protein bands had been observed in SDS-PAGE with sizes ranging from 13 kDa to 89 kDa according to treatments.

Keywords: Chlorophyll, heat stress, non-enzymatic antioxidant, protein, Solanum lycopersicum.

1. INTRODUCTION

Changing environmental conditions, such as increasing temperature, is a major concern for crop production worldwide because it greatly affects the growth, development and productivity of plants. Plants exposed to excess heat exhibit a characteristic set of cellular and metabolic responses, many of which are conserved in all organisms (Fahad et al., 2017). The high temperature induced inactivation of photosynthesis has been related to membrane damage and changes of chlorophyll (Chl) and carotenoid (Car) contents (Camejo et al., 2005).

Heat stress induces the production of ROS (reactive oxygen species), which at elevated concentrations will result in oxidative damage (Apel and Hirt, 2004) and severely reduce the yield of tomato (Tonhati et al., 2020). As sessile organisms, plants developed remarkable capabilities to rapidly respond to multiple environmental changes from which they cannot escape (Boycheva et al., 2014). Under high temperature conditions, plants accumulate different metabolites such as non-enzymatic and enzymatic antioxidants, osmoprotectants, heat shock proteins besides, different metabolic pathways and processes are activated (Foyer and Noctor, 2005). In the non-enzymatic defense system, antioxidant compounds such as Car, ascorbic acid (AsA), glutathione (GSH), and

α -tocopherol play an important role in the removal of toxic oxygen compounds (Hodges et al., 1996).

Another hypothesis put forward about adaptation to high temperature is the presence of some adaptation mechanisms; involving protein promotion and differentiated protein function (Teeri, 1980). Because many of the stress proteins are water-soluble, they probably contribute to the stress tolerance of cellular structures through hydration (Wahid and Close, 2007).

Plant responses to high temperature also vary across and within species, as well as at different developmental stages. Tomato (*Solanum lycopersicum*) is an important vegetable crop all over the world. The optimum range of daytime temperatures for tomato seedlings growth, ranges from 18 to 30°C, with an upper limit of 35°C (Abdalla et al., 2020). On the other hand, the optimum temperature during tomato plant flowering and fruit growth, ranges from 18 to 25°C (Tonhati et al., 2020). In addition, as a field and greenhouse-grown crop it is often subject to high temperatures during cultivation. Physiological observations both under field and greenhouse conditions show a variable degree of tolerance between different genotypes. Therefore early selection of plants tolerant to low or high temperatures using molecular or physiological tools can enhance the development of tolerant plants varieties. The aim of this research was to determine non-enzymatic antioxidant and protein reaction in response to heat stress in two different developmental stages (first bloom and yield stages) in three tomato cultivars.

2. MATERIALS AND METHODS

Leaves of tomato hybrid cvs. 'Çalti', 'Pembe' and 'Yaren' were used in the study. The samples were obtained in June and August from plants grown in a field under favourable conditions for tomato production, in Eskisehir, Turkey (longitude: 39°45'38"N, latitude: 30°28'47"E) during first bloom and yield period. In June, the average temperature was 21.7°C (range 7.0°C–35.2°C). In August, the average temperature was also 21.7°C (range 4.8°C–35.4°C). The third leaf from the top of the plants were collected for heat stress treatments. High temperatures were generated artificially by gradually increasing the temperature according to Arora et al. (1998) with some modifications. Briefly, leaves obtained from plants during first bloom and yield period were collected into pyrex tubes with caps closed and placed into water bath. After a 30-min habituation of the sample containing tubes in water bath adjusted to 35°C, the water temperature was inclined to 40°C in half an hour. Samples were then subjected to 40, 45, 50, 55 and 60°C temperatures with gradual increments every half an hour. Leaves collected from heat treated and control plants were analyzed for total Chl, Car, AsA, GSH contents and protein analyses.

Changes in total Chl content of tomato leaves were analyzed as described by Moran and Porath (1980). Leaf samples subjected to dimethylformamide (DMF) extraction were incubated at 4°C for 72 hours. The absorbance of supernatants was measured at 652 nm by a spectrophotometer (Perkin Elmer Lambda 25, USA). Data were expressed as mg g FW⁻¹.

The total Car content was quantified spectrophotometrically according to Lichtenthaler and Wellburn (1983) with some modifications. Fresh leaf tissues were homogenized in ice with a homogenizer (Micra D-1, Germany). Extracts were shaken at 120 rpm for three hours in the dark. Fifteen minutes later, the absorbance of the extract at 460 nm was measured.

Ascorbic acid (AsA) was determined according to Law et al. (1983) with some modifications. The assay is based on the reduction of Fe³⁺ to Fe²⁺ by AsA in acidic solution. Fe²⁺ then forms complexes with bipyridyl, giving a pink colour that absorbs at 525 nm. In extraction, 1 g of leaf samples were homogenized in 10 mL of 5% metaphosphoric acid and centrifuged at 22 000 g for 15

min. The reaction mixture for total AsA contained 0.2 mL aliquot of the supernatant, 0.5 mL of 150 mM sodium phosphate buffer (pH 7.4) containing 5 mM ethylenediamine-tetraacetic acid (EDTA), 0.1 mL of 10 mM dithiothreitol (DTT). After incubation for 15 min at room temperature, 0.1 mL 0.5 % N-ethylmaleimide was added to remove excess DTT. For the occurrence of color formation due to the amount of AsA, 0.4 mL of 10 % trichloroacetic acid (TCA), 0.4 mL of 44 % ortho-phosphoric acid, 0.4 mL of 4 % 2,2'-bipyridyl in 70 % ethyl alcohol, and 0.2 mL of 3 % FeCl₃ were added to reaction medium, respectively. The mixtures were then incubated for 40 min at 40°C and the absorbance was read in the mixtures at 525 nm using the spectrophotometer. Ranges of 0-100 µg mL⁻¹ L(+) ascorbic acid standards were used for the calculations and expressed as mg g FW⁻¹.

Glutathione (GSH) content was determined spectrophotometrically according to Ellman (1959) with some modifications. For this purpose 0.5 g of leaf sample was homogenized in 5 mL of 5% metaphosphoric acid and was centrifuged at 4 000 g for 30 min. Then, 2.5 mL of 150 mM sodium phosphate (Na-PO₄) buffer (pH 7.4) containing 5 mM EDTA and 0.5 mL of 6 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) was added to the supernatant. After the samples were incubated at room temperature for 20 min, the absorbance was read at 412 nm. Reduced glutathione standards were used for the calculations and the GSH was expressed as µg g FW⁻¹.

Total soluble protein was extracted from leaf tissues using the extraction methods described by Arora et al. (1992), with few modifications. Ground leaf tissues (1 g) were homogenized at 4°C in borate buffer [50 mM sodium tetraborate, 50 mM AsA, 1% b-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride (PMSF), pH 9.0] and insoluble polyvinylpyrrolidone (PVPP) paste made with borate buffer using a 1:5:2 (tissue: buffer: PVPP paste) extraction ratio. Samples were shaken on a gyratory shaker for 15 min at 4°C followed by centrifugation at 26 000 g for 1.5 h at 4°C. The resulting supernatant was collected and filtered through cheesecloth. Protein content was measured using the Bradford assay method (Bradford, 1976).

Proteins were precipitated according to the method described by Lim et al. (1999), by adding TCA to 1 mL of sample from the supernatant. Samples were centrifuged at 16 000 g for 30 min at 4°C, after 30 min incubation at 4°C. Protein pellets were washed three times with cold acetone and centrifuged at 16 000 g for 30 min at 4°C. After the first acetone wash, pellets were broken physically using a sealed pipette tip. Dried pellets were resuspended in SDS-PAGE sample buffer (65 mM Tris-HCl, 100 mL mL⁻¹ (v/v) glycerol, 20 mg mL⁻¹ (w/v) SDS, pH 6.8 and 50 mL mL⁻¹ b-mercaptoethanol with Bromphenol Blue). Discontinuous SDS-PAGE was performed with a PROTEAN tetra vertical electrophoresis unit (Bio-Rad, Hercules, CA, USA) using 0.04 stacking gel and 0.125 separating gel. An equal amount of total protein (30 µg) was loaded for each sample and gels were stained with Coomassie Brilliant Blue G-250.

The experiment was arranged in a randomized block design, with three replications. The data were tested with SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) and mean separation was accomplished by the Duncan test at P<0.05.

3. RESULTS AND DISCUSSIONS

Effects of heat treatments on Chl content of the leaves of three tomato cultivars are summarized in Figure 1A. When compared to control treatment, heat treatments caused a decrease in Chl content in all cultivars. The Chl content decreased gradually from control to the highest temperature (60°C). Total Chl content in the first bloom period was significantly higher than that in the yield period. The Chl content decreased gradually from ~ 6.0 mg g FW⁻¹ (in the control) to 4.9 mg g FW⁻¹ (at 50°C) in cv. 'Çaltı'. On the other hand, cv. 'Pembe' had the lowest leaf Chl content with approximately

3.5 mg g FW⁻¹. The highest relative decrease in Chl content was found in cv. ‘Yaren’ (45.8 %). At the same time, ANOVA result indicated that a significant effect of sampling period, heat stress treatments and cultivar with regard to total Chl content. Moreover, interaction between the cultivar and heat treatments was also significant (Table 1).

Data obtained from the current research revealed that total Car content of leaf tissues increased gradually from control to the highest temperature (60°C) in both sampling stages in three cultivars (Average; Control: 0.16 mg g FW⁻¹, 60°C: 0.26 mg g FW⁻¹, Figure 1B). The statistical analysis found a significant effect of heat treatments, but no significant effect of sampling period, cultivars and their interactions (Table 1).

Parallel to this research, Bhattaraie et al. (2021) reported that the Chl content of tomato decreased with temperature and duration of heat stress, suggesting damage of the photosynthetic reaction centers (Bowler et al., 1992). High temperature stress disrupts the growth and development of olive tree by inhibiting photosynthesis particularly when the temperature is above 50°C in olive plant (Cansev, 2012). Bhattarai et al.(2021) also found that heat-tolerant tomato genotypes, selected by using Chl fluorescence exhibited heat tolerance in open-field environments. The ROS induced in the chloroplast under abiotic stresses can oxidize carotenoids (Havaux, 2014). Carotenoid is a lipid-soluble antioxidant that plays multiple roles in plant metabolism, including oxidative stress tolerance (Gill and Tuteja, 2010). High light and heat stress caused significant reduction Chl content as well as increase in Car in tomato plants (Zhou et al., 2020). In addition Zhou et al. (2020) pointed out that the tomato plants developed their defense systems including Chl loss and synthesis of Car to protect themselves from multiple stresses.

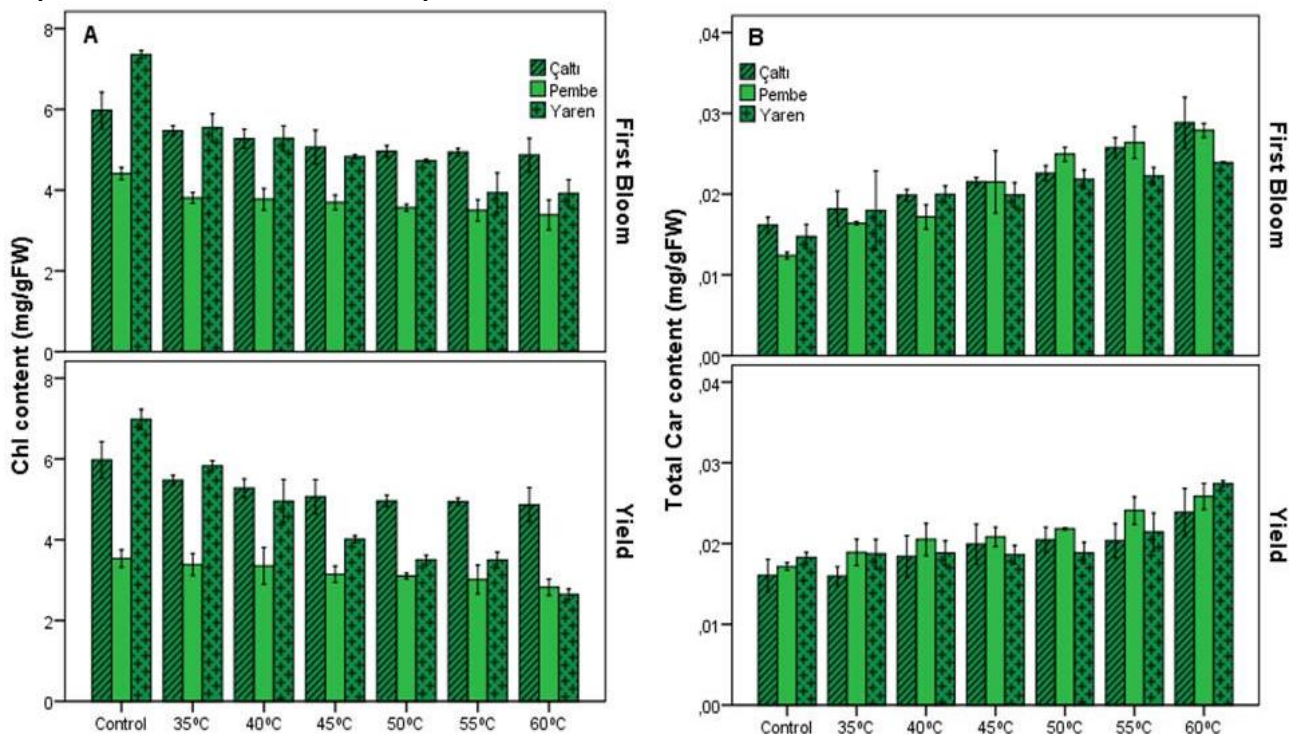


Figure 1. Effect of heat treatments on the leaf chlorophyll (Chl) (Panel A) and carotenoid (Car) (Panel B) contents of tomato cultivars in first bloom and yield stage. Vertical lines on bars indicate \pm standard errors (SE) of repetitions

Total AsA content of in the leaf of three tomato cultivars in both sampling stages is shown in Figure 2A. Heat stress treatments generally reduced the total AsA content according to control treatment in the seedling stage. However, total AsA content in three tomato cultivars was significantly higher in yield stage (~ 1.2 mg gFW⁻¹) than in first bloom stage (~ 0.6 mg gFW⁻¹). Total AsA content was generally higher in cv. 'Yaren' than in cvs. 'Pembe' and 'Çaltı'. According to the statistical analysis, it was found a significant effect of heat treatments, sampling period, cultivars and their interactions on total AsA content (Table 1).

Glutathione content values of the leaf samples fluctuated depending on temperature applications. In general, the mean GSH content in the first bloom stage (~ 936 μ g g FW⁻¹) was significantly lower than that in the yield stage (1076 μ g g FW⁻¹). Current research revealed that cv. 'Pembe' had higher GSH content than cvs. 'Yaren' and 'Çaltı' in all sampling stages (Figure 2B). The effect of sampling stage along with cultivar and heat stress treatment and their interaction on GSH content was statistically significant (Table 1).

Ascorbic acid, one of the non-enzymatic antioxidants, is a scavenger of many ROS due to its ability to donate electrons. Glutathione reacts with free radicals formed in a stress condition, preventing the sulfhydryl (SH) groups of enzymes from oxidizing. These antioxidants play a role in eliminating the damage that stress can cause (Foyer, 1993; Lamb and Dixon, 1997). Oxidative protection is an important component for determining the viability of a plant during heat stress. The present study revealed that, high temperature treatments generally reduced the total AsA content in all cultivars in the first bloom stage. However, in the yield period, although the total amount of AsA changed depending on the temperature applications, it did not show any increase or decrease trend. This may be due to the accumulation of ROS in the cell and the activation of other defense mechanisms in the removal of these ROS from the environment. Similarly, the increase in the amount of GSH because of high temperature applications may be due to an increase in the level of ROS in the environment and the cell increases the production of GSH from protective antioxidants in response to this. Researches in peas and corn have also shown that high temperature applications increase the GSH content (Nieto-Sotelo and Hu, 1986). It was determined that the contents of AsA and GSH in heat-acclimated leaves were higher than those in control at same heat stress level though the contents of AsA and GSH showed the trend of significant decrease with increase in heat stress levels. (Xu et al., 2006). In liliium, Yin et al. (2008) noted that AsA and GSH concentrations were at high levels after 10 hours of application at 37 and 42°C. However, they determined that after 10 hours of application at 47 °C, the GSH concentration was the same as the control, and the AsA concentration decreased compared to the control due to the increase in O₂⁻ and H₂O₂. (Yin et al., 2008). Ergin et al. (2016) reported that the contents of AsA and GSH did not change depending on heat stress type, temperatures, or cultivars in strawberry plants.

All the three cultivars had a significantly higher TSP content in the leaf tissues in the first bloom stage than in the yield stage (Figure 3). The data from the two sampling stages showed that the TSP content of cv. 'Yaren' (9.8 mg g FW⁻¹; Figure 3) was greater than that of cvs. 'Çaltı' (8.9 mg g FW⁻¹; Figure 3) and 'Pembe' (8.6 mg g FW⁻¹; Figure 3). Statistical analysis found a significant effect of sampling stage, cultivar, heat treatments and interaction between sampling stage, cultivar and heat treatments on TSP content (Table 1).

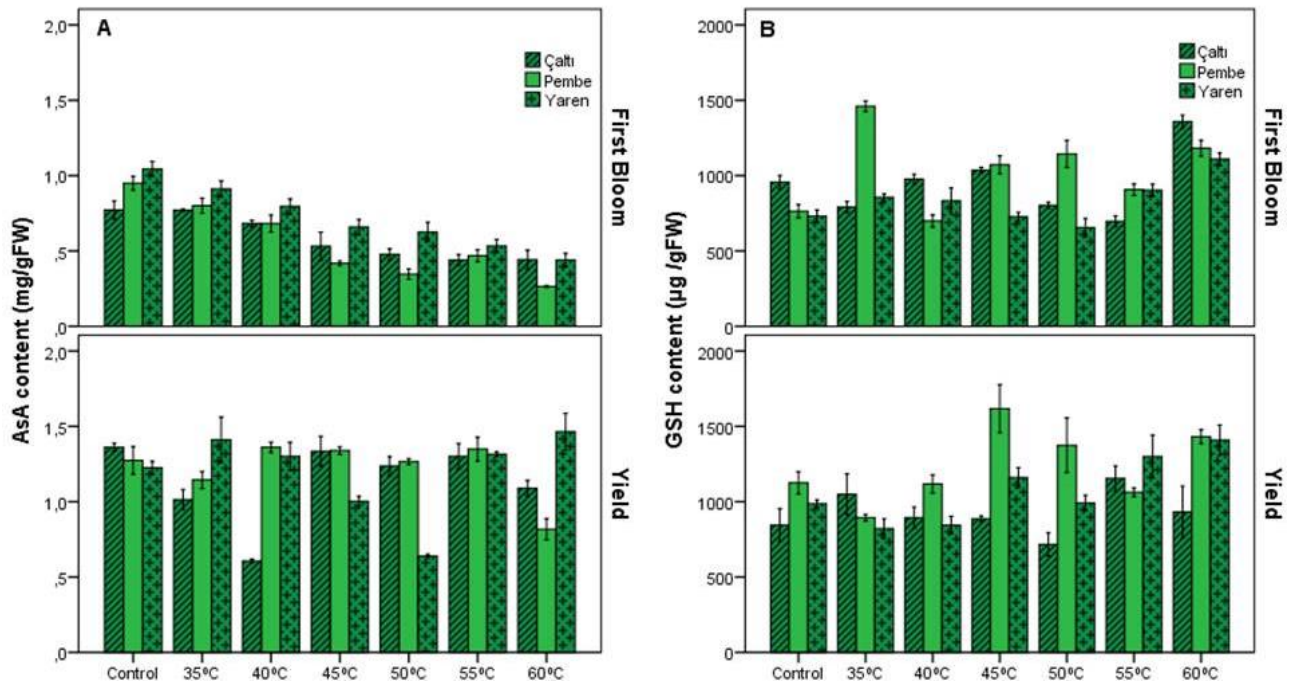


Figure 2. Effect of heat treatments on the leaf ascorbic acid (AsA) (Panel A) and glutathione (GSH) (Panel B) contents of tomato cultivars in first bloom and yield stage. Vertical lines on bars indicate \pm standard errors (SE) of repetitions

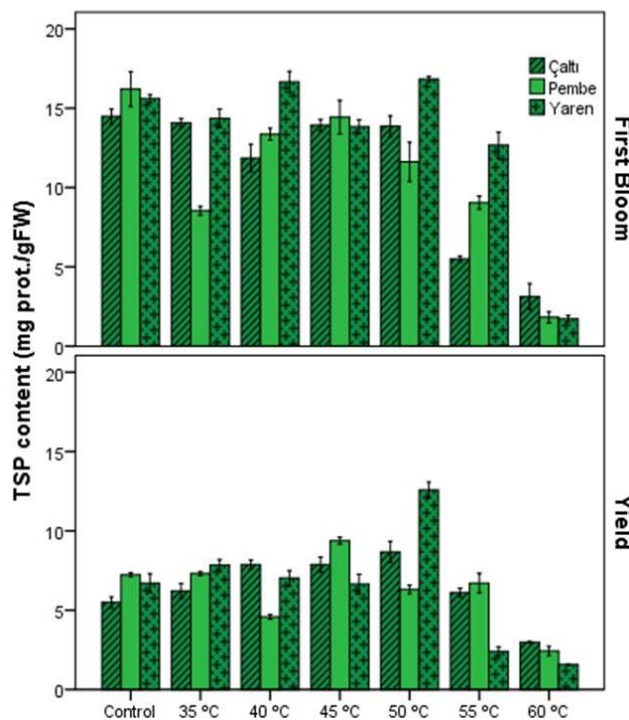


Figure 3. Effect of heat treatments on the total soluble protein (TSP) content of tomato cultivars in first bloom and yield stage. Vertical lines on bars indicate \pm standard errors (SE) of repetitions

Table 1. Results of analysis of variance (ANOVA) of period (P), cultivar (Cv.) temperature (T) and their interactions with Chl, karotenoid, AsA, GSH and TSP content in leaf tissues. Numbers represent F values relative to a significance level of 0.05

Dependent Variable	Independent Variable						
	P	Cv.	T	P×Cv.	P×T	Cv.×T	P×Cv.×T
Chl	19.921*	158.526*	35.196*	5.059*	0.756 ^{ns}	8.614*	0.699 ^{ns}
Car	1.407 ^{ns}	0.952 ^{ns}	23.880*	2.984 ^{ns}	1.822 ^{ns}	0.676 ^{ns}	0.652 ^{ns}
AsA	755.496*	7.469*	21.143*	6.674*	13.921*	7.807**	9.420*
GSH	31.247*	25.931*	13.053*	10.144*	4.991*	4.804*	6.837*
TSP	1046.712*	23.234*	226.405*	21.754*	42.798*	16.077*	13.655*

*. ^{ns}Significant and not significant at p < 0.05, respectively.

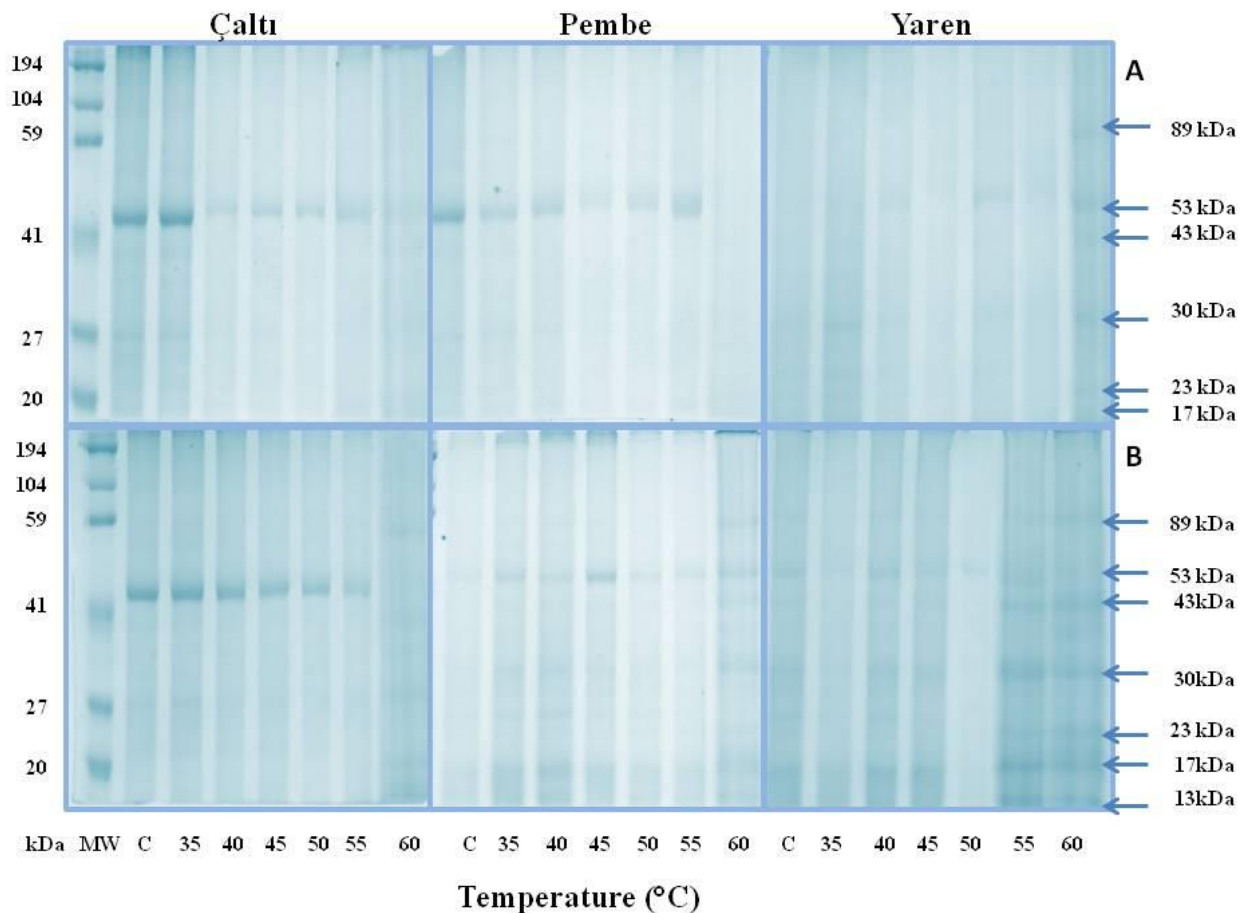


Figure 4. Effect of heat treatments on the SDS-PAGE protein profiles of tomato cultivars in first bloom (Panel A) and yield stage (Panel B). In each lane, 30 µg of protein was loaded. Molecular weight markers (MW) and the molecular mass (kDa) are shown on the left-hand side

Figure 4 shows the total protein profiles of tomato cultivars in both sampling stages depending on high temperature applications. According to the SDS-PAGE profiles, at first bloom stage, four protein bands estimated as 17, 30, 43 and 53 kDa were noted in cv. 'Çaltı'. Besides, 17, 30 and 53 kDa size of the bands have been observed in cv. 'Pembe' along with 17, 23, 30, 43, 53 and 89 kDa size of bands have been observed in cv. 'Yaren'. The 43 kDa protein band was observed from the control to 60 °C in cv. 'Çaltı'. The 30 kDa protein band disappeared after 35°C treatment in cv. 'Pembe'. On the other hand, the 43 kDa protein band was detected only at 60°C treatment at first bloom stage. According to SDS-PAGE profiles at yield stage, it is thought that the protein bands with the size of 30 and 53 kDa probably structural proteins while the size of 89 kDa protein band occur with temperature applications in tomato plants. The sizes of 13 and 17 kDa protein bands in cvs. 'Pembe' and 'Yaren', but the same band was not detected in cv. 'Çaltı' cultivar. Different from the other cultivars a protein band of 43 kDa was found in cv. 'Yaren' at yield stage (Figure 4).

The early changes caused by high-temperature stress involve in the reprogramming of signal transduction components, transcription factors and proteins associated with the metabolism of ROS under stressful conditions (Dou et al., 2015). Tokyol and Turhan (2019) reported that in 'Balkız', 'Ferasetsiz' and 'Local Genotype' green bean genotypes, the TSP content reduced and/or increased depending on the temperature and genotypes. Similarly, Turhan et al. (2015) reported that heat stress application increased the TSP content up to certain temperatures in pepper cultivars. Ergin et al. (2016) also determined that TSP content decreased, while new proteins had been synthesized depending on increased temperatures in strawberry cultivars. It was declared that new proteins are synthesized or reduced and almost complete lost in tomatoes in response to high temperature (Heckathorn et al., 1998). In addition, Turhan et al. (2015) found that strips of 7-54 kDa in the pepper plant under high temperature stress conditions and 40 kDa protein band may be associated with high temperature tolerance. The preservation of protein structures and functions under stress conditions is very important for the survival of the cell (Wang et al., 2004).

4. CONCLUSIONS

In conclusion, high temperature stress disrupts the growth and development of tomato by inhibiting photosynthesis. It has been determined that the high temperature tolerance level changes depending on the growth period of the tomato plants, there is no significant difference between the cultivars used in the study. Besides, it was found that in terms of tolerance to high temperature, non-enzymatic antioxidants AsA and GSH are effective in high temperature tolerance of tomato plants. In addition, it was also determined that TSP content decreased in parallel with the increase in temperature, whereas the synthesis of some proteins increased. Further researches on molecular basis are needed to clarify the heat stress tolerance of tomato plants.

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