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Variation in Heat Stress-Induced Some Physiological Changes and Peroxidase Activities Among Three Tomato (*Lycopersicon esculentum* Mill.) Cultivars

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Abstract

Variation in heat stress-induced some physiological changes and peroxidase (POX) activities were studied in three tomato (*Lycopersicon esculentum* Mill.) cultivars, Çaltı, Pembe and Yaren. For this purpose, the leaves were collected from tomato plants at the first bloom and yield stages. The leaves were subjected to heat stress treatments in water bath at 35, 40, 45, 50, 55 and 60 °C with gradual increments every half an hour. The leaves were then analysed for ion leakage, loss of turgidity, soluble peroxidase (S-POX) and cell wall-bound peroxidase (CWB-POX) activities. In general, effects of heat stress on the variables studied were significant. Results revealed that ion leakage and loss of turgidity were increased parallel to the temperatures. In addition, loss of turgidity values were higher in the yield stage than those in the first bloom stage. Considering the POX activities, the S-POX activity was greater in the first bloom stage than in the yield stage in all cultivars. Moreover, the highest and the lowest enzyme activity were detected in cvs. Yaren and Pembe, respectively. In contrast to S-POX activity, the CWB-POX activity was greater in the yield stage than in the first bloom stage in cvs. Çaltı and Pembe. The highest CWB-POX activity was detected in cv. Çaltı while the lowest activity was detected in cv. Pembe. Data also indicated that, generally CWB-POX activity was high in the samples in response to heat stress treatments.

Keywords: Tomato, heat stress, ion leakage, loss of turgidity, peroxidase

Üç Domates (*Lycopersicon esculentum* Mill.) Çeşidinde Yüksek Sıcaklık Stresinin Teşvik Ettiği Bazı Fizyolojik Değişiklikler ve Peroksidaz Aktivitelerinde Değişim

Özet

Çaltı, Pembe ve Yaren domates (*Lycopersicon esculentum* Mill.) çeşitlerinde sıcaklık stresinin teşvik ettiği bazı fizyolojik değişiklikler ve peroksidaz (POX) aktivitelerindeki farklılıklar belirlenmiştir. Bu amaçla domates bitkilerinden ilk çiçeklenme ve verim dönemlerinde yaprak örnekleri toplanarak, su banyosunda sıcaklık kademeli olarak arttırılarak 35, 40, 45, 50, 55 ve 60 °C sıcaklıklarda, her sıcaklık kademesinde 30 dakika tutulmuştur. Yaprak örneklerinde iyon sızıntısı, turgor kaybı, çözünebilir peroksidaz (S-POX) ve hücre duvarı peroksidaz (CWB-POX) aktiviteleri belirlenmiştir. Genel olarak sıcaklık stresinin incelenen parametreler üzerindeki etkileri önemli bulunmuştur. Sonuçlar, iyon sızıntısı ve turgor kaybı değerlerinin sıcaklıklara paralel olarak arttığını göstermiştir. Bunun yanı sıra turgor kaybı değerleri verim döneminde ilk çiçeklenme dönemine göre daha yüksek bulunmuştur. POX aktiviteleri değerlendirildiğinde, S-POX aktivitesinin tüm çeşitlerde ilk çiçeklenme döneminde verim dönemine oranla daha yüksek olduğu tespit edilmiştir. Diğer taraftan en yüksek ve en düşük S-POX aktivitesi sırasıyla Yaren ve Pembe çeşitlerinde olmuştur. CWB-POX aktivitesi ise S-POX aktivitesinin aksine verim döneminde ilk çiçeklenme dönemine oranla daha yüksek bulunmuştur. En yüksek CWB-POX aktivitesinin Çaltı, en düşük CWB-POX aktivitesinin ise Pembe çeşidinde olduğu saptanmıştır. Veriler aynı zamanda CWB-POX aktivitesinin genellikle sıcaklık stresi uygulamasına bağlı olarak arttığını göstermiştir.

Anahtar Kelimeler: Domates, sıcaklık stresi, iyon sızıntısı, turgor kaybı, peroksidaz

Introduction

Temperature is one of the most important environmental factors for survival of plants. With global warming, high temperature is predicted to be one of the limiting factors for cultivation of tomato and other plants in the future.

Heat stress causes multifarious, and often adverse, alterations in plant growth, development, physiological processes, and yield (Levitt, 1980; Hasanuzzaman et al., 2013). Acclimation, a process that increases stress tolerance, may occur in response to mild non-lethal stress. Many of the changes that appear during acclimation to heat stress are reversible, but if the stress is too great, irreversible changes can occur and these can lead to death (Gulen and Eris, 2003). The increased solute leakage, as an indication of decreased cell membrane thermostability, has long been used as an indirect measure of heat-stress tolerance in various plant species (Chen et al., 1982; Arora et al., 1998, Gulen and Eris, 2003; Cansev, 2012). Electrolyte leakage is influenced by plant/tissue age, sampling organ, developmental stage, growing season, degree of hardening and plant species (Wahid et al., 2007).

One of the major consequences of heat stress is the excess generation of reactive oxygen species (ROS), which leads to oxidative stress (Anderson, 2002). Peroxidases are involved in many physiological processes in plants, involving responses to biotic and abiotic stresses and the biosynthesis of lignin. They are also involved in the scavenging of ROS, which are partially reduced forms of atmospheric oxygen, highly reactive, and capable of causing oxidative damage to the cell. Peroxidases can be a source of hydrogen peroxide (H_2O_2) but also are capable of scavenging it (Gaspar et al., 1982; Vicuna, 2005). POX enzyme has been related to the appearance of physiological injuries caused in plants by thermal stress, and its activity was enhanced by high temperature stress (Chaitanya et al., 2002; Mazorra et al., 2002).

Tomato (*Lycopersicon esculentum* Mill.) is an economically important vegetable species in both Turkey and the World. Tomato has been cultivated in open field and greenhouses in Turkey. Tomato plants have been frequently exposed to high temperature during their different developmental periods. Moreover tomato production under high temperature conditions reduces product quality and yield (Mansour et al., 2009). High temperature is now a major concern for crop production and approaches for sustaining high yields of crop plants under high temperature stress are important agricultural goals (Hasanuzzaman et al., 2013). Therefore, researches concentrated on the high temperature metabolism in plant will be very important. In this respect, our objective in this study was to investigate the effects of the relationship between heat stress and one of the antioxidant enzymes, POX. The experiment was also conducted to examine the effect of heat stress on changes in electrolyte leakage in cellular level and loss of turgidity in three tomato cultivars.

Materials and Methods

Leaves of tomato cultivars cv."Çaltı", "Pembe" and "Yaren" were used in the study. The samples were obtained in June and August from plants during first bloom and yield period were from an field in Eskisehir. The monthly mean, maximum and minimum temperatures from January to December in 2012 at the experimental area are shown in Figure 1. High temperatures were generated artificially by gradually increasing the temperature as described previously 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. Samples that were obtained at each treatment temperature were analysed for ion leakage, loss of turgidity, and S-POX and CWB-POX activities.

Ion leakage tests were done as previously described by Arora et al. (1992). Briefly, leaf disks of 1 cm diameter from each treatment group were placed into test tubes containing 10 mL of deionized water. The samples were then subjected to vacuum infiltrated at -0.15 MPa for 5 min to allow uniform diffusion electrolyte and incubated on a shaker at 250 rpm at room temperature (24 ± 1°C) for 4 hours before electrical conductivity of each sample was measured by conductivity meter (YSI, USA). Leaf discs were killed in the same solution by autoclaving and total conductivity was measured at room temperature. Electrolyte leakage was expressed as the percentage of total ions present in the tissue. For obtaining turgid weight, 1 cm leaf discs were floated on distilled water in a petri dish for 4 h at room temperature. After incubation, leaf discs were removed from the petri dish, surface-blotted, and immediately weighed. For oven drying, leaf discs were put in a new dry petri dish with lid and placed in an oven at 70 °C for 48.h. After incubation leaf discs were weighed (Barr and Weatherley, 1962). The activities of S-POX and CWB-POX in the leaf tissues

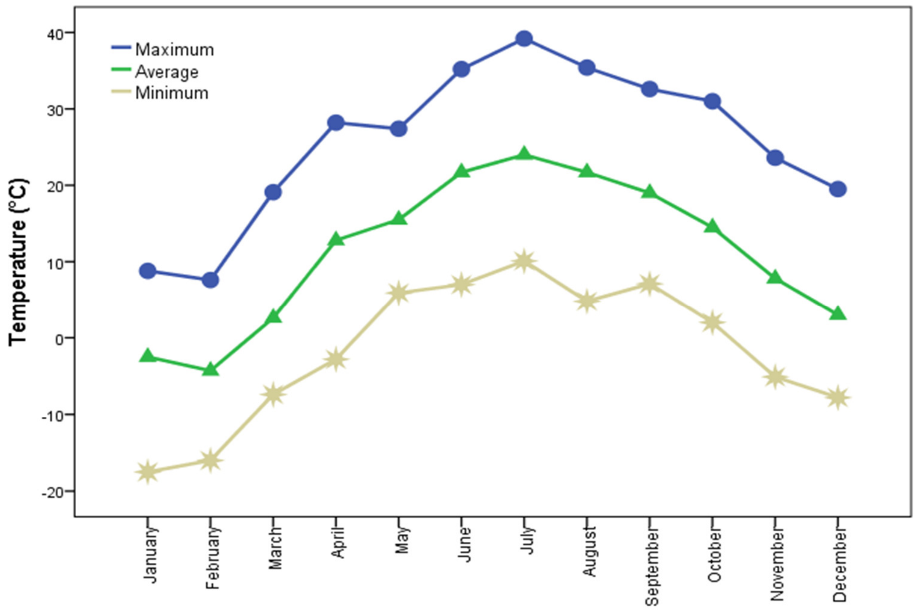

Figure 1. Monthly mean, maximum and minimum temperatures from January to December in 2012 at the experimental site.

were determined spectrophotometrically according to Andrews et al. (2000). In short, liquid N_2-frozen tissue (1.0 g) was extracted using a mortar and pestle with 2.0 mL 1.0% (v/v) polyvinylpyrrolidone (PVPP) and 5 mL 100 mM potassium-phosphate buffer, pH 7.0, containing 0.1 mM EDTA and 0.1% (v/v) Triton X-100. The extract was filtered through four layers of cheesecloth and centrifuged at 15,000 x g for 20 min at 4ºC. The supernatant was used to assay for S-POX activity. The CWB pellet was washed four-times with the same buffer, and incubated on ice in 5 mL 1 M NaCl for 5 h. The CWB suspension was then dialysed against distilled water at 4°C for 48 h and centrifuged at 10,000 x g for 30 min at 4°C.The supernatant was used for enzyme assays. POX activity was also measured according to Andrews et al. (2000). Aliquots of 20 – 100 µL of tissue extract were added to 2 mL of a reaction mixture containing 100 mM Na acetate-citrate buffer, pH 6.0, and 5 µg mL^{-1} TMBHCl. The reaction was started by adding 10 µL 6% (v/v) H_2O_2, and incubation was carried out for 30 min at 25°C. To stop the reaction, 0.5 mL of 0.6 M H_2SO_4 was added and the optical density was determined in a UV/VIS spectrophotometer (Lambda EZ201; Perkin Elmer, Norwalk, CT, USA) at 450 nm. POX activity was expressed by reference to a standard curve using horseradish peroxidase (Sigma-Aldrich).

The experiment was arranged in a randomized block design, with three replications. The data were tested with SPSS 20.0 for Windows SPSS (Inc., Chicago, IL, USA) and mean separation was accomplished by the Duncan test at P<0.05.

Results

We found that exposure to high temperatures caused varying rates of injury in tomato depending on the sampling period, cultivar and heat stress treatment (Figure 2A, Table 1). In general, ion leakage was greater in the first bloom stage at each temperature treatment compared to that in the yield stage ($p<0.05$). Difference with regard to percent ion leakage between cultivars was more prominent starting with 55°C treatment in the yield stage. Rate of ion leakage was significantly ($p<0.05$) greater in cvs. Çaltı and Yaren compared with that in cv. Pembe with 55 and 60°C treatments. The greatest ion leakage at 55°C and 60°C treatment (94.98% and 96.72%, respectively) was observed in tomato cultivars. Statistical analysis revealed a significant effect of sampling period, cultivar and high temperature treatments, and a significant interaction of sampling period and high temperature treatments with regard to percent ion leakage (Table 1).

The changes in loss of turgidity values in the leaf tissues are shown in figure 2B. According to average values, loss of turgidity was significantly greater in yield stage than in first bloom stage in all cultivars. The highest loss of turgidity value in the first bloom stage was detected in cv. Yaren (18.02 %) while the lowest loss of turgidity value in the first bloom stage was detected in cv. Çaltı (15.11 %). On the other hand, the highest (25,88 %) and the lowest (20.90 %) loss of turgidity values in the first bloom stage was detected in cv. Çaltı (15.11 %). On the other hand, the highest (25,88 %) and the lowest (20.90 %) loss of turgidity values in the yield period were detected in cv. Çaltı and cv. Yaren, respectively. Although percent loss of turgidity did not show enormous differences among tomato cultivars, our data suggest that cv. Çaltı showed a better adaptation to high temperatures. Statistical analysis revealed a significant effect of sampling period, cultivar, heat stress treatment and a significant interaction of sampling period, cultivar and high temperature treatments with regard to percent loss of turgidity (Table 1).

S-POX enzyme activity was significantly ($p<0.05$) greater in the first bloom stage than in the yield stage in all cultivars. S-POX enzyme activity was higher in either cv. Yaren compared with cvs. Çaltı and Pembe in both first bloom and the yield stages (Fig. 3A). The highest and the lowest S-POX enzyme activity in the first bloom stage were observed in cv. Yaren (7,148 units/mg protein) and cv. Pembe (3.409 units/ mg protein) cultivars, respectively. The highest and the lowest S-POX enzyme activity in the yield stage were observed in cv. Çaltı (1.598 units/mg protein) and cv. Yaren (1,341 units/mg protein), respectively. Statistical analysis revealed a significant effect of sampling period, cultivar, and the interaction of sampling stage, cultivar and heat treatments on S-POX activity. However there was no significant effect heat treatment on S-POX activity (Table 1).

CWB-POX enzyme activity was significantly greater in the yield stage than in the first bloom stage generally in all cultivars (Fig. 2B). The highest and the lowest CWB-POX activity were detected in cv. Yaren (0.14 units/mg protein) and in cv. Pembe (0.038 units/mg protein) in the first bloom stage, respectively. In addition, the highest and the lowest CWB-POX enzyme activity were detected in cv. Çaltı (0.187 units/mg protein) and cv. Yaren (0.066 units/mg protein) in the yield stage, respectively. CWB-POX activity generally was high in the samples in response to heat stress treatments. Statistical analysis revealed a significant effect of sampling period, cultivar, heat treatments and the interaction of sampling period, cultivar and heat treatment on CWB-POX activity (Table 1).

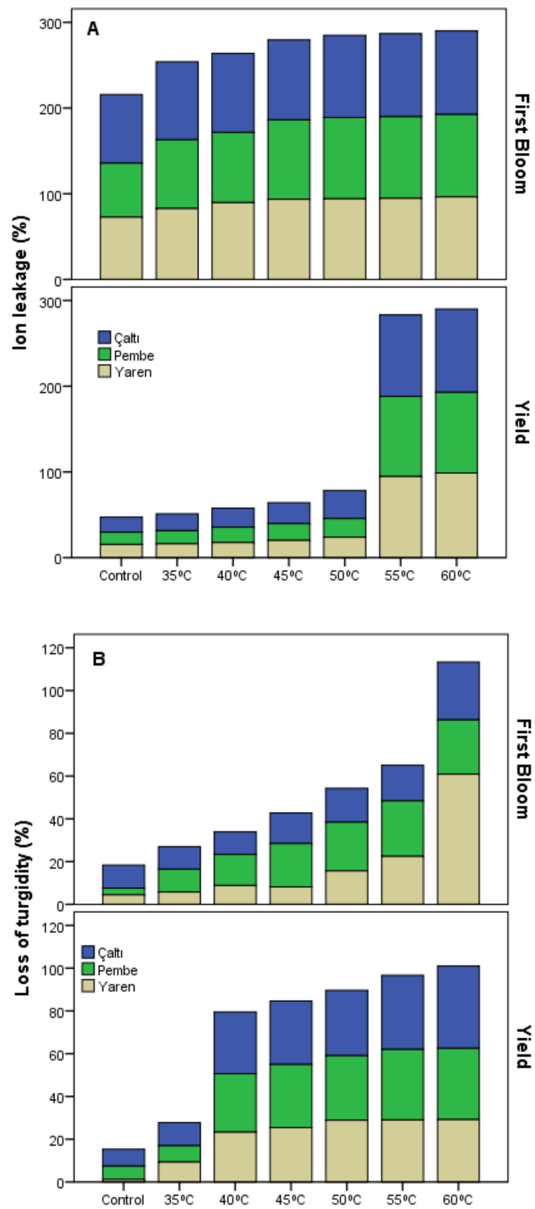

Figure2. The effects on ion leakage (Panel A) and loss of turgidity (Panel B) of tomato cultivars with respect to exposure to high temperatures. Control samples not subjected to high temperature treatment.

Table 1. Results of the analysis of variance (ANOVA) of sampling period (P), cultivar (Cv.), heat stress treatment (T) and their interactions for loss of turgidity, ion leakage, S-POX and CWB-POX activities.

Dependent Variable	Independent Variable						
	P	Cv.	T	PxCv.	PxT	Cv.xT	PxCv.xT
Ion leakage	3450.458*	14.298*	461,853*	0.330^{ns}	253.890*	1.010^{ns}	1.374^{ns}
Loss of turgidity	261.397*	3.566*	356.29*	30.971*	54.452*	23.850*	30.561*
S-POX activity	427.077*	42.471*	1.550^{ns}	55.824*	2.700*	7.266*	4.240*
CWB-POX activity	176.736*	40.977*	20.136*	295.160*	18.628*	41.337*	15.779*

Notes: Numbers represent F-values at 5% level
**,^{ns} Significant and not significant at P≤0.05*

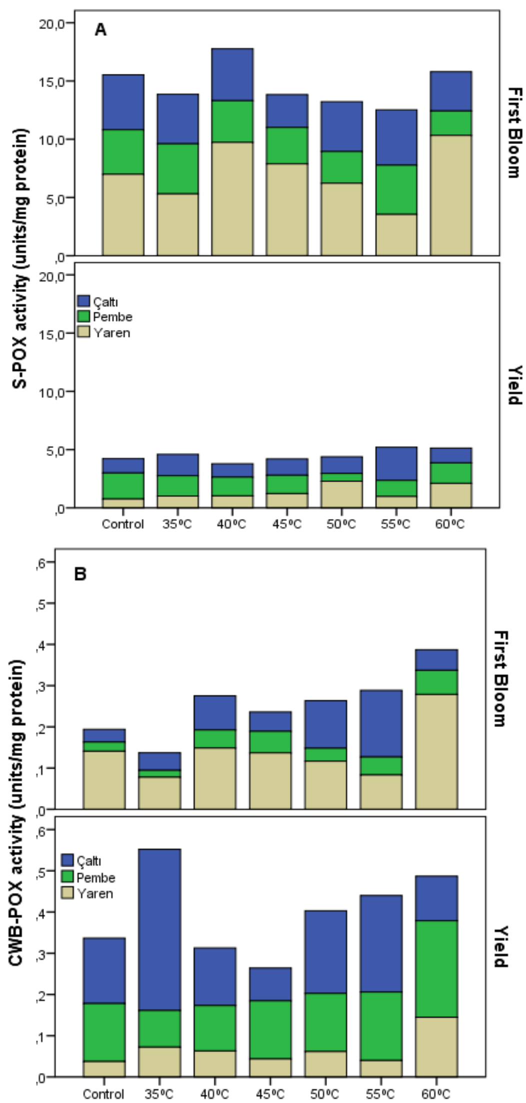

Figure 3. The effects on S-POX (Panel A) and CWB-POX activities (Panel B) of tomato cultivars with respect to exposure to high temperatures. Control samples not subjected to high temperature treatment.

Discussion

We investigated, in the present study, changes in percent ion leakage in leaf tissues of tomato cultivars cvs. Çaltı, Pembe and Yaren in the first bloom period and the yield period with respect to high temperature treatments. Although percent ion leakage did not show enormous differences among tomato cultivars, our data suggest that cv. Pembe showed a better adaptation to high temperatures. The ion leakage of strawberry plants has been shown to increase with high temperature treatment in strawberry plant (Gulen and Eris, 2003; Kesici et al., 2013). In addition, in good accord with the present finding, high temperature stress was shown to enhance ion leakage in tomato (Wang et al., 2006), *Lilium longiflorum* (Yin et al., 2008), small reddish bean (Aydoğan and Turhan, 2013), wheat (Gupta et al., 2013) and *Brassica juncea* (Kumar et al., 2013).

It has been reported that the measurement of RWC and loss of turgidity are useful indicators of the water balance in a plant (Else and Atkinson, 2010). Primarily, high temperatures cause increases in transpiration, and this change leads to an increase the loss of turgidity (Cansev, 2012; Kesici et al., 2013). Reduction of loss of turgidity due to increases in heat stress was observed strawberry and olive leaves (Gulen and Eris, 2003; Cansev, 2012; Kesici et al., 2013).

Although total peroxidase activity of plant extracts is a stress marker, results are not clear, because of the high amount of isoenzymes that may be differentially regulated (Mika et al., 2010). The peroxidase reaction is unspecific towards the type of stress. For example, Gulen and Eris (2004) showed that total and specific POX activities were significantly increased by high temperatures in strawberry plants. In addition, S-POX activity was increased in onion bulbs by low temperature stress (Benkeblia and Shiomi, 2004). On the other hand, S-POX activity decreased in cadmium treated *Brassica juncea* (L.) czern seedlings while treatment with cadmium resulted in an increase in ionically bound CWP activity in roots of the seedlings (Verma et al., 2008). Ros-Barceló et al (1988) reported that POXs have been found soluble in the apoplastic fluid or linked by ionic or covalent bounds to cell wall components. CWB- POX activity was induced by low temperature in sweet cherry cultivars grafted on different rootstocks (Cansev and Kesici, 2013). However, the association of CWB-POX activity with heat stress treatments has not yet been understood. In agreement, a previous study reported that CWB- POX activity was induced by low temperature suggesting that it could serve as an antioxidant enzyme to protect mitochondria from chilling-induced oxidative stress in maize (Prasad et al., 1995). Therefore, our present data showing that CWB-POX activity was enhanced during heat stress treatment are in good accord with previous reports.

Conclusion

Our data indicated that In general, effects of heat stress on the variables studied were significant. The results revealed that ion leakage and loss of turgidity were increased parallel to the temperatures. In addition, loss of turgidity values were higher in the yield stage than those in the first bloom stage. Considering the POX activities, the S-POX activity was greater in the first bloom stage than in the yield stage in all cultivars. Data also indicated that, generally CWB-POX activity was high in the samples in response to heat stress treatments.

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