http://dergipark.gov.tr/trkjnat
Trakya University Journal of Natural Sciences, 18(2): 97-104, 2017
ISSN 2147-0294, e-ISSN 2528-9691
DOI: 10.23902/trkjnat.289527

Research Article/Araştırma Makalesi

CHANGES IN PHOTOSYNTHETIC PIGMENTS, ANTHOCYANIN CONTENT AND ANTIOXIDANT ENZYME ACTIVITIES OF MAIZE (*Zea mays* L.) SEEDLINGS UNDER HIGH TEMPERATURE STRESS CONDITIONS

Elif YÜZBAŞIOĞLU[1*], Eda DALYAN[1], Ilgın AKPINAR[2]

[1]Department of Botany, Faculty of Science, Istanbul University, Süleymaniye, Istanbul
[2] Institute of Sciences, Istanbul University, Vezneciler, Istanbul
*Corresponding author: e-mail: aytamka@istanbul.edu.tr

Received (Alınış): 3 February 2017, Accepted (Kabul): 31 May 2017, Online First (Erken Görünüm): 26 July 2017, Published (Basım): 15 December 2017

Abstract: This study was performed in order to determine the effects of gradually increasing temperatures on maize, which belongs to the C_4 plant group. 20 day old seedlings were exposed to increasing heat stress (25/20, 30/25, 35/30, 40/35, 45/40°C at 16/8 photoperiods) for 5 days. The first temperature treatment (25/20°C) was used as control. Stress injury was measured in terms of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), chlorophyll (*a* and *b*), carotenoid and anthocyanin contents and maximum quantum efficiency of photosystem II (Fv/Fm). MDA and H_2O_2 levels were found to significantly increase at high temperatures (35, 40, 45°C). Chlorophyll content was observed to be highest at 35°C and a decrease was determined at 40 and 45°C. *F*v/*F*m was found to decrease at 40 and 45°C. Carotenoid and anthocyanin contents dramatically increased under high temperature stress. In addition, significant increases were determined in the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) under high temperature (45°C), while peroxidase (POX) and glutathione S-transferase (GST) activities showed no change. Treatments above 35°C triggered high temperature stress in maize seedlings. The results of this study showed that temperatures above 35°C lead to stress effects on photosynthesis and induced enzymatic and non-enzymatic antioxidant activity in maize seedlings.

Key words: Anthocyanin, antioxidant enzymes, heat stress, maize.

Mısır (*Zea mays* L.) Fidelerinde Yüksek Sıcaklık Stresi Koşullarında Fotosentetik Pigmentler, Antosiyanin İçeriği ve Antioksidan Enzim Aktivitelerindeki Değişiklikler

Özet: Bu çalışmada, C_4 tipi fotosentez yapan mısır bitkisinde giderek artan sıcaklığın etkilerinin çalışılması amaçlanmıştır. 20 günlük fideler 5 gün boyunca giderek artan (25/20, 30/25, 35/30, 40/35, 45/40°C 16/8 fotoperiyot) sıcaklık stresine maruz bırakılmıştır. Uygulanan ilk sıcaklık (25/20°C) kontrol grubu olarak kullanılmıştır. Stress hasarı, malondialdehit (MDA), hidrojen peroksit (H_2O_2), klorofil (a ve b), karotenoid ve antosiyanin içeriği ve fotosistem II'nin maksimum kuantum verimi (Fv/Fm) ile belirlenmiştir. MDA ve H_2O_2 seviyelerinin yüksek sıcaklıkta (35, 40, 45°C) önemli ölçüde arttığı bulunmuştur. Klorofil içeriğinin 35°C'de en yüksek olduğu gözlenmiştir ancak 35°C sıcaklık ile karşılaştırıldığında 40 ve 45°C uygulanan sıcaklıklarda klorofil içeriğinde azalma belirlenmiştir. Fv/Fm 40 ve 45°C sıcaklık uygulamasında düşüş göstermektedir. Karotenoid ve antosiyanin içeriği yüksek sıcaklık stresi altında önemli ölçüde artmaktadır. Ayrıca, yüksek sıcaklık da (45°C) superoksit dismutaz (SOD), katalaz (CAT), askorbat peroksidaz (APX) ve glutatyon reduktaz (GR) enzim aktiviteleri belirgin bir şekilde artış gösterirken, peroksidaz (POX) ve glutatyon-S-transferaz (GST) enzim aktivitesinde değişiklik gözlenmemiştir. Mısır fidelerinde 35°C'nin üzerinde bir sıcaklık uygulanması yüksek sıcaklık stresine neden olmaktadır. Bu çalışmanın sonucunda, mısır fidelerinde 35°C'nin üzerindeki sıcaklıkların fotosentez üzerinde stress etkisine yol açtığı ve enzimatik ve enzimatik olmayan antioksidan aktiviteyi teşvik ettiği ortaya konmuştur.

Anahtar kelimeler: Antioksidan enzimlar, antosiyanin, mısır, sıcaklık stresi.

Introduction

Heat waves or extreme temperatures are increasingly observed climate conditions and expected to become more intense and frequent in near future compared today (Hatfield & Prueger 2015). Intergovernmental Panel on Climatic Change (IPCC) (2014) reported that hot days and nights in global scale were often observed while number of cold days and nights reduced. Extreme temperature conditions may last for short-term durations of a few days with temperature increases of over 5°C above the normal temperatures (Hatfield & Prueger 2015). Extreme temperatures observed during summer season were reported to have a negative impact on plant growth,

development and productivity (Bita & Gerats 2013). For instance, when higher plants are exposed temperature values at least 5°C above their optimal growth temperature values, their organelles and cytoskeleton and membrane stability are affected as a result of cellular and metabolic responses of the plants to survive in increased temperature conditions (see Bita & Gerats 2013). The photosynthetic apparatus in plants is heat sensitive and in particular, chlorophyll biosynthesis, net photosynthetic rate, Rubisco activity and PSII center are the primary targets of thermal damage in plants (Sinsawat *et al.* 2004). Due to the damage of the photosynthesis processes in plants caused by heat stress, photoinhibition occurs, leading to accumulation of reduced electron acceptors and thereby acceleration of the formation of reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), which is associated with oxidative damage (Cui *et al.* 2006). At the cellular level, high temperatures can result in excessive accumulation of ROS, which in turn can trigger lipid peroxidation and damage pigments, nucleic acid and proteins (Wang *et al.* 2014). Plants have developed mechanisms protecting them against the damage caused by ROS. These mechanisms consist of scavenging the ROS by non-enzymatic antioxidants, such as carotenoids, anthocyanins, and mobilization of an enzymatic antioxidant system, which includes catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), peroxidase (POX) and glutathione S-transferases (GSTs) (Almeselmani *et al.* 2006, W. Chen *et al.* 2012). Carotenoids protect photosystems and chlorophyll molecules by reacting with lipid peroxidation products and scavenging singlet oxygens (Wahid 2007). Anthocyanins are highly effective antioxidants for excessive accumulation of stress-induced ROS and the protection of osmotic balance (Wahid 2007, Bita & Gerats 2013). Metalloenzyme SOD (EC 1.15.1.1), the most effective intracellular enzymatic antioxidant, catalyzes the partitioning of the superoxide radicals to molecular oxygen and H_2O_2 (Gill & Tuteja 2010). Catalase (EC 1.11.1.6), a tetrameric heme-containing enzyme, has one of the fastest turnover rates for all enzymes, and catalytic rate for one catalase molecule is nearly 6 million molecules of H_2O_2 altered to water and oxygen per minute (Gill & Tuteja 2010, Choudhury *et al.* 2013). Ascorbate peroxidase (EC 1.11.1.11) has showed high specificity for ascorbate as the electron donor and plays an essential role in sweeping hydrogen peroxide in chloroplast (Shigeoka *et al.* 2002). Glutathione reductase (EC 1.6.4.2) is a potential enzyme of the ascorbate-glutathione cycle and remove H_2O_2 by catalyzing the reduction of glutathione (Gill & Tuteja 2010). Peroxidase (EC 1.11.1.7) is another heme-containing enzyme that performs H_2O_2-detoxifying activities. GSTs (EC 2.5.1.18) are known to be responsible for detoxification of xenobiotics but can also play an important role as antioxidants by removing lipid peroxidation end products (Dalton *et al.* 2009).

Maize is a highly efficient plant in suitable environmental and growth conditions but is also very susceptible to drought and heat. An average of 15- to 20% of the potential world maize production is lost every year due to temperature related climatic stresses (J. Chen *et al.* 2012). Although C_4 plants such as maize are more stable at higher temperatures, net photosynthesis is usually inhibited when the temperature of maize leaves exceed roughly 38°C (Crafts-Brandner & Salvucci 2002, Coşkun *et al.* 2011). Sinsawat *et al.* (2004) reported that when maize was subjected to an increase in temperature from 25°C to 35°C for 20 min in the dark, short-term inhibition occurred in the rate of photosynthesis, and permanent damage resulted at temperatures above 45°C. In the same study, when ΦPSII and photochemical quenching factor (qP) and the efficiency of open reaction centers (F`v/F`m) were slowly reduced between 35 and 45°C, the maximum quantum efficiency of PSII (Fv/Fm) in maize leaves clearly decreased at temperatures above 45°C (Sinsawat *et al.* 2004). In another study in which maize was exposed to long-term high temperature and combined high temperature and water deficit, plant biomass accumulation was reported to decrease under all treatments (Perdomo *et al.* 2015). High temperature (35°C) treatment was shown to positively affect vegetative plant growth in maize but decreased ear expansion and reduced cob extensibility (Suwa *et al.* 2010). Kumar *et al.* (2012) treated maize and rice genotypes to high temperature stress (35/30, 40/35, 45/40°C) for 11 days. The results of this study showed that inhibition of growth, decreased leaf water and chlorophyll content, increased electrolyte leakage, higher H_2O_2 levels, elevated MDA content and increased antioxidant enzyme activities (SOD, CAT, APX, GR) were observed at temperatures above 35/40°C.

Although many studies reported data on the effect of long-term heat stress on maize photosynthesis efficiency and yields, little is known about the short-term extreme temperature effect on maize seedlings. In this study we focused on the effects of short-term gradually rising temperatures (increase of 5°C every day, from 25°C to 45°C) on maize seedlings. We also investigated the changes in photosynthetic pigment, chlorophyll fluorescence, anthocyanin content, lipid peroxidation, H_2O_2 content and antioxidant enzyme activity in response to the gradually rising temperature in maize leaves

Materials and Methods

Plant Material and Treatment

Maize (*Zea mays* L.) seeds were obtained from MayAgro Seed Corporation (71-May-69). The experiments were performed in a plant growth chamber (VB 0714, Bioline, Vötsch Industrietechnik, Germany; internal dimensions: 970x750x1400cm; lighting intensity: 450mmol $m^{-2}s^{-1}$ @ 200mm). Seeds were imbibed in deionized water for 24 hours at room temperature. Three seeds were sown in plastic pots (120mm diameter and 110mm depth) each containing a perlite. The seedlings were grown for 20 days in the growth chamber at conditions of 16-8h photoperiod, 25\20 °C and 60% humidity. Maize seedlings were

irrigated every three days with 1\4 Hoagland solution (Hoagland & Arnon 1950). Heat stress treatments were performed using 20 pots and 4 pots were used for each temperature treatment. Heat stress was achieved by increasing the temperature everyday by 5°C from 25°C to 45°C. Plants were sampled 24h after being exposed to 25°C, 30°C, 35°C, 40°C and 45°C, just before temperature rose to next level. The photoperiod and the light intensity were kept constant during the experiments. During heat stress treatments, plants were irrigated twice daily to prevent drought effects. Fresh leaves were used for the analyses.

Determination of Photosynthetic Pigments

Leaves were homogenised in 100% acetone and the samples were centrifuged at 3000xg for 15 min at 4°C. Leaf extacts were measured spectrophotometrically at 661.6, 644.8, and 470nm. Chlorophyll a, b, total chlorophyll, and carotenoid contents of the extracts were determined in µg/ml by employing the method of Lichtenthaler (1987).

Chlorophyll Fluorescence Analysis

The maximum quantum efficiency of photosystem II (PSII) photochemistry (Fv/Fm) was used as chlorophyll fluorescence parameter. Fv/Fm was performed by using the LI-6400XT Portable Photosynthesis and Fluorescence System (Li-Cor Inc., Licoln, USA). The Fv/Fm ratio was calculated as (Fm −Fo)/Fm. Fm and Fo are the maximum and basal fluorescence yields. Leaves were dark-adapted for 20 min prior to fluorescence measurements.

Determination of Anthocyanin

Anthocyanin content was determined by using the method of Mancinelli (1990). Plant samples (0,5g) were extracted in 3ml methanol-HCl (1% HCl, v/v) and the homogenates were kept at 3-5ºC for 2 days with occasional shakings. The extracts were filtered, and anthocyanin and chlorophyll contents were measured at 530nm and 657nm, respectively. The anthocyanin content (µg/ml) was measured using the formula A530-A657 and subtracting chlorophyll absorption.

Determination of Malondialdehyde (MDA) Content

Fresh leaf samples (0.5g) were extracted in 10mL of 0.25% thiobarbituric acid (TBA) in 10% trichloroacetic acid (TCA). The extracts were heated at 95°C for 30min and quickly cooled on ice. The samples were centrifuged at 5000xg for 10min. The absorbance was measured at 532nm and 600nm using a spectrophotometer. The level of MDA was calculated as $nmolg^{-1}$ of fresh weight using the extinction coefficient of $155mM^{-1}cm$ (Jiang & Zhang 2001).

Determination of Hydrogen peroxide (H_2O_2) Content

Hydrogen peroxide was assayed by the method of Velikova *et al.* (2000). Fresh leaf tissue (0.5g) was finely homogenized with 0.1% (w/v) TCA. The homogenate was centrifuged at 12,000xg for 15min and 0.5ml phosphate buffer (pH 7.0) and 1ml potassium iodide were added on the supernatant (0.5ml). Its absorbance was recorded at 390nm after using a Epoch 2 Microplate Reader. H_2O_2 content was estimated by using H_2O_2 standart curve.

Determination of Antioxidant enzymes

Leaf samples (0.5g) were homogenized in 3mL of 50mM potassium phosphate buffer (pH 7.0) including %1PVPP and 1mM EDTA and centrifuged at 13,000×g for 40min 4°C. The supernatant was used for protein and enzyme activity determinations. All spectrophotometric analyses were conducted on Epoch 2 Microplate Reader. Bradford (1976) method was used in determination of protein concentration using bovine serum albumin as a standard. The activity of ascorbate peroxidase (EC 1.11.1.11) was determined by monitoring the decrease in absorbance at 290nm, as ascorbate was oxidized (Nakano & Asada 1981). Catalase (CAT; EC 1.11.1.6) was analyzed according to the method described by Bergmeyer (1970). Total superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed by inhibition of photochemical reduction of NBT at 560nm (Beuchamp & Fridovich, 1971). Glutathione reductase (EC 1.6.4.2) activity was measured according to Foyer & Halliwell (1976). Peroxidase (EC 1.11.1.7) activity was determined by method of Herzog & Fahimi (1973). GST activity was assayed with 1-chloro-2,4-dinitrobenzene (CDNB) by a modified method of Carmagnol *et al.* (1981).

Statistical analysis

All experimental data were analyzed using the mean ± standart error values of at least five replicates. A one-way ANOVA followed post hoc analysis with the Tukey's Multiple Comparison test was applied to test the significance of differences between the mean values. All analyzes were performed on GraphPad Prism version 5.2 for windows (GraphPadSoftware, San Diego, CA).

Results and Discussion

Effect of Heat Stress on Photosynthetic Characteristics

Photosynthesis is one of the most sensitive mechanisms to high temperature stress in plants (Sinsawat *et al.* 2004). High temperature stress impacts the structural and functional construction of chloroplasts, and the damage on chloroplasts can cause temporary or permanent reduction of photosynthetic efficiency and chlorophyll accumulation (Cui *et al.* 2006). To examine the effects of high temperature on maize photosynthesis, we measured chlorophyll *a* and *b* content, chlorophyll *a/b* ratio, total chlorophyll/carotenoid ratio and maximum quantum efficiency of photosystem II (Fv/Fm). The results showed that chlorophyll *a* and *b* increased when the temperature reached 35°C but when the temperature reached 40 and 45°C, chlorophyll *a* was reduced in comparison to 35°C. Chlorophyll *b* was found to be more stable up to the highest temperature of 45°C. The chlorophyll *a/b* ratio decreased following the temperature increase from 25 to 45°C. Carotenoid content was found to increase with increasing temperature and the highest level was observed at 45ºC. Total chlorophyll/carotenoid

ratio significantly decreased at temperatures above 35°C (Table 1). When maize leaves were exposed to increasing temperatures, chlorophyll *a* experienced faster degradation than chlorophyll *b*. Chlorophyll *b* is present only in the pigment antenna system, but Chlorophyll *a* is found in both the pigment antenna and the reaction centers of photosystems I and II. The chlorophyll *a/b* ratio is the most important finding for light adaptation of the photosynthetic apparatus and the functional pigment equipment (Lichtenthaler *et al.* 1981). When chlorophyll *a/b* ratio decreases, it may be explained as an enlargement of the antenna system of PS II. These chlorophyll results suggest that maize is resistant to short-term heat waves (Table 1). Other aspects of chlorophyll were also found to be indicators of heat tolerance of maize under heat stress treatments. Almeselmani *et al.* (2006) indicated that heat tolerant wheat cultivars maintained comparatively higher chlorophyll content under increasing temperature in late and very late plantings. Asensi-Fabado *et al.* (2013) reported that chlorophyll content in three labiatea species was stable when the plants were exposed to increasingly higher temperatures. Maximum quantum efficiency of photosystem II (Fv/Fm) in dark adapted leaves was more stable in maize seedlings that received high temperature stress treatment. However, the increasing temperature, particularly the 45°C treatment, resulted in reduction of Fv/Fm compared to the control (25°C). High temperature stress affected the Fv/Fm causing a structural and functional deficiency of the photosynthetic apparatus (Cui *et al.* 2006). The reduction in Fv/Fm under heat stress showed in particular that the PSII reaction center was injuried and induced to photoinhibition (Cui *et al.* 2006, Efeoğlu & Terzioglu 2009). Crafts-Brandner & Salvucci (2002) reported that Fv/Fm was relatively insensitive to leaf temperatures up to 42.5°C. Many studies have indicated that PSII is the most heat-sensitive component of photosynthesis (Crafts-Brandner & Salvucci 2002, Sinsawat *et al.* 2004, Cui *et al.* 2006, Efeoglu & Terzioglu 2009).

Effect of Heat Stress on Hydrogen Peroxide and Lipid Peroxidation

The highly toxic ROS production significantly increases and induces peroxidation of membrane lipid under stress events and H_2O_2 and MDA concentrations have been widely used as criterion to determine heat injury in plants. Hydrogen peroxide content increased nearly 2-folds at 30, 35 and 40°C in comparison to control (25°C), and the highest H_2O_2 content was observed at 45°C. The results also showed that the MDA concentration in maize leaves increased after 35, 40 and 45ºC treatments (Fig. 1), compared to the control (25ºC), and the MDA level was more stable at 30°C treatment. The finding that H_2O_2 content started to increase even at 30°C showed that it was the most sensitive to temperature changes, but no change occurred in MDA level at 30°C. Both H_2O_2 and MDA contents were highest at 45°C (Fig. 1). All these findings suggest that short-term and gradually increasing temperature initially triggers H_2O_2 production in maize leaves. Heat stress induces generation and reactions of activated oxygen species (AOS) including singlet oxygen, superoxide radical, hydrogen peroxide and hydroxyl radical cause of cellular injury (Wahid *et al.* 2007). AOS generate the autocatalytic peroxidation of membrane lipids and pigments thereby causing the loss of membrane semi-permeability and functions (Asthir 2015). The resulting damage seen in plants after exposure to heat stress was described as the injury of photosynthesis and cell membrane fluidity (Choudhury *et al.* 2013). Heat stress mainly effects membrane fluidity, especially photosynthetic and mitochondrial membranes (Asthir 2015). Membrane damages are known as stress parameters used in determination of level of lipid destruction. It is known that lipid peroxidation products are formed from polyunsaturated precursors that include small hydrocarbon fragments such as MDA (W. Chen *et al.* 2012, Asthir 2015). Savicka & Škute (2010) reported that ROS production and MDA content increased in various development stages of wheat that underwent long-term high temperature treatment. Furthermore, the enhancement of lipid peroxidation and H_2O_2 in apple leaves was shown to occur in response to high temperature (Ma *et al.* 2008). In our study, no changes were observed in leaf water content during high temperature treatment, due to the fact that plants were irrigated twice daily to prevent drought effects.

Table 1. Changes in chlorophyll *a* and *b* content, chlorophyll *a/b* ratio, carotenoid content, total chlorophyll/carotenoid ratio and Fv/Fm (maximum quantum efficiency of photosystem II) after gradually increasing temperature values the maize leaves were exposed to. The means ±SE of five replicates were given. Different letters denote statistically significant differences by Tukey's Multiple Comparison test ($P < 0.05$) among all treatments respectively.

Heat treatment	Chl a (µg ml^{-1})	Chl b (µg ml^{-1})	Chl a/b	Carotenoid (µg ml^{-1})	Total chl/car	Fv/Fm
25 °C	61.70±0.57d	13.57±0.24d	4.55±0.09a	14.64±0.15e	5.14±0.6b	0.79±0.007a
30 °C	77.98±1.90b	21.44±0.42c	3.64±0.12b	16.91±0.44d	5.88±0.07a	0.70±0.02b
35 °C	90.81±1.71a	26.05±0.70a	3.49±0.09b	21.06±0.66b	5.55±0.08c	0.73±0.009b
40 °C	76.82±0.60b	24.08±0.55b	3.19±0.07c	19.38±0.43c	5.21±0.15b	0.70±0.017b
45 °C	69.25±0.65c	25.57±1.19a	2.71±0.15d	23.20±0.69a	4.09±0.14d	0.63±0.08c

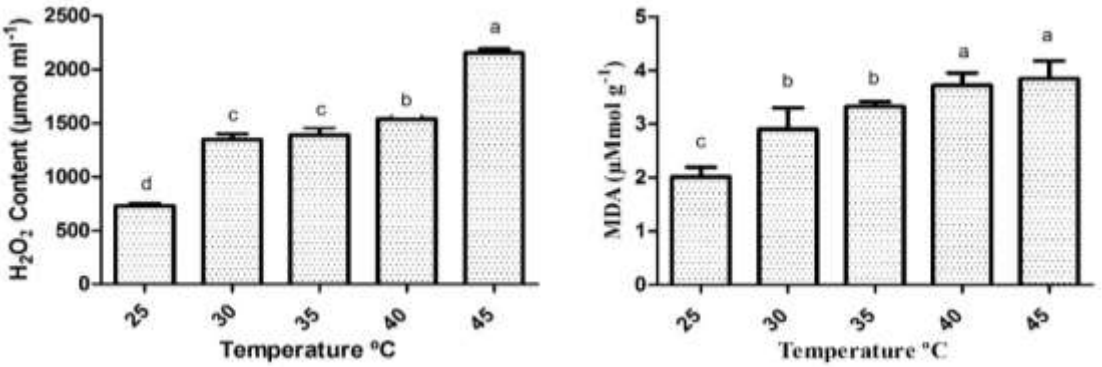

Fig. 1. Changes in hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents in maize leaves at gradually increasing temperature treatment. Heat stresses were exposed to a steady temperature increase of 5°C every day from 25°C to 45. Data shown corresponds to means ± SE of five replicates. Different letters denote statistically significant differences by Tukey's Multiple Comparison test (P <0.05) among all treatments respectively.

Effect of Heat Stress on Anthocyanin Level

In this study, gradually increasing temperature caused accumulation of anthocyanin in maize. Although a minor increase occurred at 30 and 35°C, compared to the control, the increase at 40 and 45°C was approximately 3-folds compared to the control (Fig. 2). Anthocyanins are secondary metabolites that govern the colors of plant tissues and are generally produced in the cytoplasm before being transported into the vacuole. As a polyphenol, anthocyanin is an effective antioxidant and scavenger of ROS and therefore plays an important role in environmental stress (Shao *et al.* 2007). Some studies reported that anthocyanin is sensitive to light and heat alteration (Gould *et al.* 2002, Shao *et al.* 2007). Shao *et al.* (2007) determined that anthocyanins had a protective role in high temperature injury and triggered antioxidative capacity under high temperature stress in *Arabidopsis*. Mori *et al.* (2007) found that anthocyanin accumulation in red-wine grapes decreased under high temperatures.

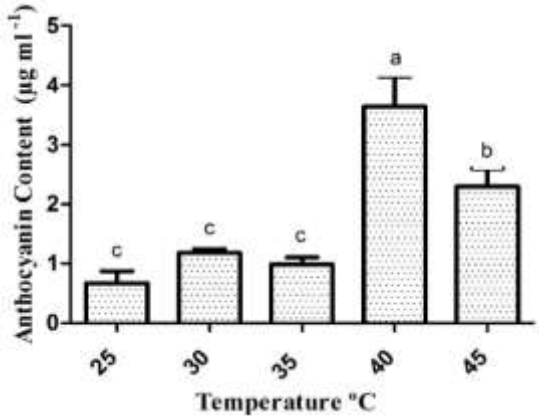

Fig. 2. Changes in anthocyanin content in maize leaves at gradually increasing temperatures. Data shown corresponds to means ±SE of five replicates. Different letters denote statistically significant differences by Tukey's Multiple Comparison test (P <0.05) among all treatments respectively.

Effect of Heat Stress on Antioxidant Enzyme Activity

Plants stimulate various enzymatic systems to alleviate the harmful effects of ROS under high temperature stress. To better understand this process, we examined the activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR) and glutathione S-transferase (GST) (Fig. 3) in maize leaves under gradually rising temperature. SOD, the first defence mechanism of the antioxidant system, catalyses the dismutation of O_2^- to H_2O_2 and O_2. APX, GR and CAT and POD are able to scavenge H_2O_2 through different mechanisms (Gill & Tuteja 2010). Our results showed that antioxidant enzymes responded differently to gradually increasing temperature stress. To cope with the oxidative stress, SOD, POX, CAT and GR enzyme activities increased, and APX and GST stabilized when the temperature reached 30°C. All enzyme activities noticeably reached their lowest levels at 35°C, which was considered as heat acclimatization. When temperature was increased from 35 to 45°C, SOD, CAT, APX and GR activities were positively triggered, but no significant increase occurred in GST and POX activities. SOD, CAT, GR and APX activities were at highest levels when the temperature reached 45°C (Fig. 3). The antioxidant enzyme activities suggest that maize plants trigger an effective system for detoxifying active oxygen species when temperature increases from 25°C to 45°C. It was also found in our study that heat treatment enhanced activities of SOD, CAT, GR and APX in maize leaves. Various studies have been conducted on the changes in antioxidant enzyme activity in plants under high temperature stress (Gür *et al.* 2010, He & Huang 2012, Wang *et al.* 2014, Ergin *et al.* 2016). The activities of SOD, APX, CAT, GR, and POX were shown to increase significantly at all stages of growth in wheat cultivar C306 (heat-tolerant), while the PBW343 (heat-sensitive) genotype was shown to have a significantly reduced CAT, GR, and POX activities in response to high temperature stress (Almeselmani *et al.* 2006). Kumar *et al.* (2012) compared the responses of *Oryza sativa* and *Zea mays* to varying degrees of temperature stress (35/30, 40/35, 45/40°C) and found that CAT, APX and GR enzyme activities were higher in maize plants compared to rice but that no changes occurred in SOD at 45/40°C.

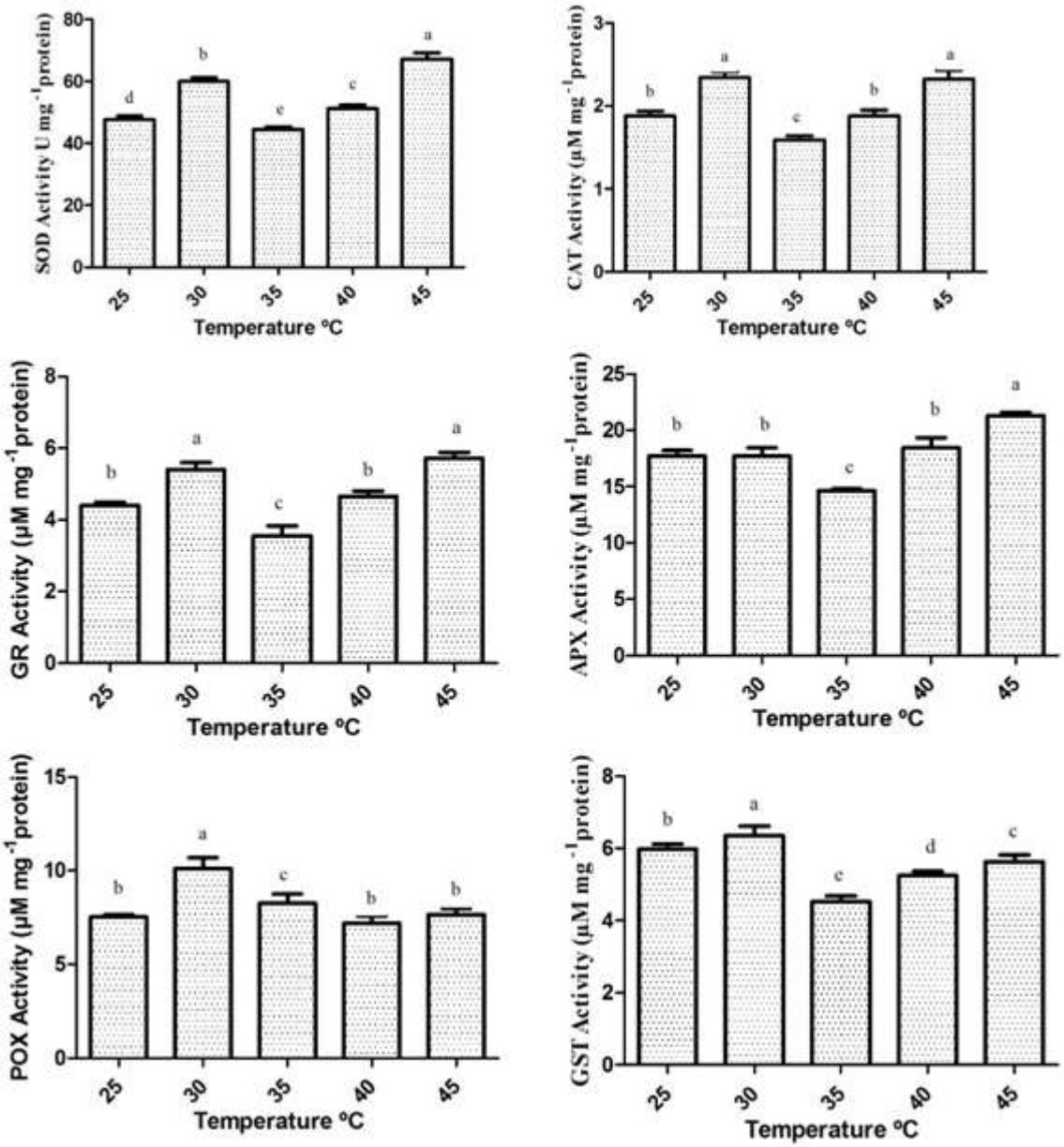

Fig. 3. Changes in antioxidant enzymes activities (SOD, CAT, GR, APX, POX and GST) at gradually increasing temperature. Data shown corresponds to means ±SE of five replicates. Different letters denote statistically significant differences by Tukey's Multiple Comparison test ($P < 0.05$) among all treatments respectively.

Conclusion

The results of this study provide evidence that maize seedlings represent a high tolerance to gradually increasing temperatures (25-45°C). Improved thermo-tolerance may relate to high anthocyanin and carotenoid content besides antioxidant enzymes activity may improve thermo-tolerance. Data presented in this study also contributes to better understanding of physiological process in maize exposed to increased temperatures.

Acknowledgement

This work was supported by Scientific Research Projects Coordination Unit of Istanbul University (Project numbers: 41364, FBA-2016-3745).

References

1. Almeselmani, M., Deshmukh, P.S., Sairam, R.K., Kushwaha, S.R. & Singh T.P. 2006. Protective role of antioxidant enzymes under high temperature stress. *Plant Science*, 171: 382-388.
2. Asensi-Fabado, M.A., Oliván, A. & Munné-Bosch, S. 2013. A comparative study of the hormonal response to high temperatures and stress reiteration in three Labiatae species. *Environmental and Experimental Botany*, 94: 57-65.
3. Asthir, B. 2015. Protective mechanisms of heat tolerance in crop plants. *Journal of Plant Interaction*, 10(1): 202-210.
4. Bergmeyer, N.1970. *Methoden der enzymatischen, Analyse*. Akademie Verlag, Berlin, Vol. 1, 636-647.

5. Beuchamp, C. & Fridovich, I 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44: 276-287.

6. Bita, C.E. & Gerats, T. 2013. Plant tolerance to high temperature in a changing environment: scientific fundamental sand production of heatstress-tolerant crops. *Frontiers Plant Science*, 273(4): 1-18.

7. Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248-254.

8. Carmagnol, F., Sinet, P.M., Rapin, J. & Jerome, H. 1981. Glutathione-S-transferase of human RBCs; assay, values in normal subjects and in two pathological circumstance. Hyperbilirubinaemia and impaired renal function. *International Journal of Clinical Chemistry*, 117: 209-217.

9. Chen, J., Xu, W., Velten, J., Xin, Z. & Stout, J. 2012. Characterization of maize inbred lines for drought and heat tolerance. *Journal of Soil and Water Conservation*, 67(5): 354-364.

10. Chen, W., Cen, W., Chen, L., Di, L., Li, Y. & Guo, W. 2012. Differential Sensitivity of Four Highbush Blueberry (*Vaccinium Corymbosum* L.) Cultivars to Heat Stress. *Pakistan Journal of Botany*, 44(3): 853-860.

11. Choudhury, S., Panda, P., Sahoo, L. & Panda, S.K. 2013. Reactive oxygen species signaling in plants under abiotic stress. *Plant Signalling & Behavior*, 8(4): e23681 DOI: 10.4161/psb.23681.

12. Crafts-Brandner, S.J. & Salvucci, M.E. 2002. Sensitivity of photosynthesis in a C4 plant, maize, to heat stress. *Plant Physiology*, 129: 1773-1780.

13. Cui, L., Li, J., Fan, Y., Xu, S. & Zhang, Z. 2006. High temperature effects on photosynthesis, PSII functionality and antioxidant activity of two *Festuca arundinacea* cultivars with different heat susceptibility. *Botanical Studies*, 47: 61-69.

14. Coşkun, Y., Coşkun, A., Demirel, U. & Özden, M. 2011. Physiological response of maize (*Zea mays* L.) to high temperature stress. *Australian Journal of Crop Science*, 5(8): 966-972.

15. Dalton, D.A., Boniface, C., Turner, Z., Lindahl, A., Kim, H.J., Jelinek, L., Govindarajulu, M., Finger, R.E. & Taylor, C.G. 2009. Physiological roles of glutathioneS-transferases in soybean root nodules. *Plant Physiology*, 150: 521–530.

16. Efeoglu, B. & Terzioglu, S. 2009. Photosynthetic responses of two wheat varieties to high temperature. *EurAsian Journal of BioSciences*, 3: 97-106.

17. Ergin, S., Gülen, H, Kesici, M., Turhan, E., İpek, A. & Köksal, N. 2016. Effects of high temperature stress on enzymatic and nonenzymatic antioxidants and proteins in strawberry plants. Turkish Journal of Agriculture and Forestry, 40: 908-917.

18. Foyer, C.H. & Halliwell, B. 1976. The presence of glutathione and glutathione reductase in chloroplast: a proposed role in ascorbic acid metabolism. *Planta*, 133: 21-25.

19. Gill, S.S. & Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, 48: 909-930.

20. Gould, K.S., Mckelvie, J. & Markham, K.R. 2002. Do anthocyanins function as antioxdants in leaves? Imaging of H_2O_2 in red and green leaves after mechanical injury. *Plant, Cell & Environment*, 25: 1261-1269.

21. Gür, A., Demirel, U., Özden, M., Kahraman, A. & Çopur, O. 2010. Diurnal gradual heat stress affects antioxidant enzymes, proline accumulation and some physiological components in cotton (*Gossypium hirsutum* L.). *African Journal of Biotechnology*, 9: 1008–1015.

22. Hatfield, J.L. & Prueger, J.H. 2015. Temperature extremes: Effect on plant growth and development. *Weather and Climate Extremes*, 10: 4-10.

23. He, Y. & Huang, B. 2012. Differential responses to heat stress in activities and isozymes of four antioxidant enzymes for two cultivars of *Kentucky bluegrass* contrasting in heat tolerance. *Journal of American Society for Horticultural Science*, 135: 116-124.

24. Herzog, V. & Fahimi, H. 1973. Determination of the activity of peroxidase. *Analytical Biochemistry*, 55: 554–562.

25. Hoagland, D.R. & Arnon, D.I. 1950. The water culture method for growing plants without soil. *California Agricultural Experiment Station*, 347: 32.

26. IPCC 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

27. Jiang, M. & Zhang, J. 2001. Effect of abscisic acid on active oxygen species, antioxidative defense system and oxidative damage in leaves of maize seedlings. *Plant and Cell Physiology*, 42: 1265-1273.

28. Kumar, S. Gupta, D. & Nayyar, H. 2012. Comparative response of maize and rice genotypes to heat stress: status of oxidative stress and antioxidants. *Acta Physiologia Plantarum*, 34: 75-86.

29. Lichtenthaler, H.K. 1987. Chlorophyll and carotenoids: pigments of photosynthetic biomembranes, in: L. Packer, R. Douce (Eds.), *Methods in Enzymology*. Plant Cell Membranes, Academic Press, New York, pp. 350-382.

30. Lichtenthaler, H.K., Buschmann, C., Döll, M., Fietz, H.J., Bach, T., Kozel, U., Meier, D. & Rahmsdorf, U. 1981. Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. *Photosynthesis Research*, 2: 115-141.

31. Ma, Y.H., Ma, F.W., Zhang, J.K., Li, M.J., Wang, Y.H. & Liang, D. 2008. Effects of high temperature on activities and gene expression of enzymes involved in ascorbate–glutathione cycle in apple leaves. *Plant Science*, 175: 761-766.

32. Mancinelli, A.L. 1990. Interaction between light quality and light quantity in the photoregulation of anthocyanin production. *Plant Physiology*, 92: 1191-1195.

33. Mori, K., Goto-Yamamoto, N., Kitayama, M. & Hashizume, K. 2007. Loss of anthocyanins in red-wine

grape under high temperature. *Journal of Experimental Botany*, 58 (8): 1935-1945.

34. Nakano, Y. & Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*, 22: 867-880.

35. Perdomo, J.A., Conesa, M.À., Medrano, H., Ribas-Carbó, M. & Galmés, J. 2015. Effects of long-term individual and combined water and temperature stress on the growth of rice, wheat and maize: relationship with morphological and physiological acclimation. *Physiologia Plantarum*, 155: 149-165.

36. Savicka, M. & Škute, N. 2010. Effects of high temperature on malondialdehyde content, superoxide production and growth changes in wheat seedlings (*Triticum aestivum* L.). *Ekologija*, 56: 26-33.

37. Shao, L., Shu, Z., Sun, S-L., Peng, C-L., Wang, X-J. & Lin, Z-F. 2007. Antioxidation of anthocyanins in photosynthesis under high temperature stress. *Journal of Integrative Plant Biology*, 49(9): 1341-1351.

38. Shigeoka, S, Ishikawa, T, Tamoi, M., Miyagawa, Y, Takeda, T, Yabuta, Y, Yoshimura, K. 2002. Regulation and function of ascorbate peroxidase İsoenzymes. *Journal of Experimental Botany*, 53(372): 1305-1319.

39. Sinsawat, V., Leipner, J., Stamp, P. & Frachebound, Y. 2004. Effect of heat stress on the photosynthetic apparatus in maize (*Zea mays* L.) grown at control or high temperature. *Enviromental and Experimental Botany*, 52: 123-129.

40. Suwa, R., Hakata, H., Hara, H., El-Shemy, H.A., Adu-Gyamfi, J.J., Nguyen, N.T., Kanai, S., Lightfoot, D.A., Mohapatra, P.K. & Fujita, K. 2010. High temperature effects on photosynthate partitioning and sugar metabolism during ear expansion in maize (*Zea mays* L.) genotypes. *Plant Physiology and Biochemistry*, 48: 124-130.

41. Velikova, V., I. Yordanov A. & Edreva A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective roles of exogenous polyamines. *Plant Science*, 151: 59-66.

42. Wahid, A. 2007. Physiological implications of metabolite biosynthesis for net assimilation and heat-stress tolerance of sugarcane (Saccharum officinarum) sprouts. Journal of Plant Research, 120: 219-228.

43. Wahid A, Gelani S., Ashraf M. & Foolad MR. 2007. Heat tolerance in plants: an overview. *Enviromental and Experimental Botany*, 61: 199-223.

44. Wang, C., Wen, D., Sun, A., Han, X., Zhang, J., Wang, Z. & Yin, Y. 2014. Differential activity and expression of antioxidant enzymes and alteration in osmolyte accumulation under high temperature stress in wheat seedlings. *Journal of Cereal Science*, 60: 653-659.