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DROUGHT INDUCED PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES IN *Solanum lycopersicum* GENOTYPES DIFFERING TO TOLERANCE

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ABSTRACT

Drought stress is one of the most serious abiotic stresses that cause reduction in plant growth, development and yield in many parts of the world. The plants have developed different morphological, physiological and biochemical mechanisms to withstand drought stress. The present study investigated different levels (S1: 100% of field capacity – Control; S2: 50% of field capacity –moderate stress; S3: 0% of field capacity-severe stress) of drought stress on oxidative damages and variations in antioxidants in the two tomato genotypes Tom-163 (drought-sensitive), Tom-143 (drought-tolerant) to elucidate the antioxidative protective mechanism governing differential drought tolerance. The shoot fresh weight, shoot height, leaf number and area, relative water content (RWC) were reduced with different level of drought stress. However, this reduction clearly occurred in Tom-163 (sensitive). Antioxidative enzyme activities such as superoxide dismutase, catalase, ascorbate peroxidase and glutation reductase had a greater increase in tolerant genotypes (Tom-143) than in sensitive ones (Tom-163). The level of lipid peroxidation was measured by estimating malondialdehyde content. Lipid peroxidation increased with rising drought level in both genotypes although Tom-143 was the least affected when compared with the Tom-163. Total phenolic and flavonoid contents increased in tomato genotypes under S2 and S3 conditions. The highest total phenolic and flavonoid contents were attained in Tom-143 subjected to S3 treatment. These results indicated that antioxidant defense systems, osmolytes and secondary metabolites play important roles in tomato during drought stress.

Key words: CAT, flavonoid, MDA, proline, SOD, tomato

INTRODUCTION

Abiotic stresses such as salinity, drought, chiling and oxidative adversely affect plant growth and development [Latif et al. 2016]. Water shortage is predicted as the most severe environmental problem for the 21st century and drought is a major abiotic factor that limits crop production [Yuan et al. 2010].

Acclimation of plants to water deficit is the result of different events, which lead to adaptive changes in plant growth and physio-biochemical processes, such as changes in plant structure, growth rate, issue osmotic potential and antioxidant defenses [Anjum et al. 2011]. Environmental stresses such as drought enhance the generation of reactive oxygen species (ROS). Free ROS attack biological structures, damaging DNA, prompting the oxidation of amino acids and proteins, and provoking lipid peroxidation. Two

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classes are found in general for the non-enzymatic antioxidants; lipid soluble membrane associated antioxidants for example, α-tocopherol and β-carotene, and water soluble reductants for example, glutathione, phenolics and ascorbate. Ascorbate peroxidase (APX), superoxide dismutase (SOD) and glutathione reductase (GR) compose enzymatic antioxidants and they are thought to search for H_2O_2 in chloroplast and mitochondria. Catalase (CAT) and peroxidase (POD) are the other enzymatic antioxidants and are able to remove H_2O_2, and can neutralise or scavenge oxyintermediates and free radicals [Jaleel et al. 2009]. Key enzymes involved in the detoxification of ROS are named SOD, CAT, peroxidase (POD), APX and other enzymes implicated in the Halliwell and Asada cycle (ascorbate–glutathione pathway). Under stress condition that enhanced activity of almost all these enzymes.

Superoxide radicals that emerge as result of stress in the plant tissues are transformed into hydrogen peroxide (H_2O_2) by the SOD enzyme [Dixit et al. 2001, Mittiova et al. 2002]. The accumulation of H_2O_2, which results from the canalization reaction of the SOD enzyme and is a powerful oxidant, is prevented by the ascorbate-glutathione cycle. The hydroxyl radical (OH), which is very reactive and the most toxic oxide, can react with all macromolecules without discrimination. SOD and CAT, by combining their actions can prevent or decrease the formation of this oxide. Even though the particular scavengers are not fully known of the single radical oxygen or the hydroxyl radical, it is thought that SOD functions in removal via chemical reaction [Jaleel et al. 2009]. In the defence against intracellular antioxidants SOD and GSH work together and SOD prevents the radical mediated chain oxidation of GSH, thus helping GSH in its role as a free radical scavenger physiologically, without the accompaniment of oxidative stress [Asada 1999, Jaleel et al. 2009]. It was observed that with continued stress conditions SOD enzyme activity, which acts by decreasing the oxidative oxygen species derived from stress, continued to increase. Even though the linearity of increased stress duration and the increase of SOD activity are concurrent, it was shown that genotypes with more tolerance are superior in this area. The enzyme CAT changes oxidative stress induced reactive oxygen derivatives, like H_2O_2, into water and molecular oxygen [Kusvuran et al. 2016]. Catalase, found mostly in glyoxisomes of lipid-storing tissues in plants, contains a tetrameric heme that catalyses the conversion of hydrogen peroxide, produced from the β-oxidation of fatty acids, into water and oxygen [Lopez-Huertas et al. 2000]. The GR and APX enzymes, which are a part of the defence mechanism of tolerant genotypes against salt, drought, and chilling stress, are generally effective in the reduction of hydrogen peroxide to water in chloroplasts and mitochondria, thereby detoxifying them. Ascorbate peroxidase is one of the most important antioxidant enzymes of plants that detoxify H_2O_2 using ascorbate for reduction. Different isoforms of APX are active in chloroplasts, cytosol and microsomes. In the ascorbate-glutathione cycle, APX reduces H_2O_2 into water by oxidizing ascorbate into monodehydroascorbate (MDHA) which is then converted into ascorbate via the MDHA reductase enzyme, thus 2 MDHA molecules are changed into MDHA and dehydroascorbate (DHAR) as a non-enzymatic side product in unequal amounts. Subsequently, the reduction of DHA occurs and ascorbate is produced by the action of dehydroascorbate reductase (DHAR) and GR. DHAR can then convert GSH into GSSG which then is reduced back into GSH by GR [Kusvuran et al. 2013]. Due to APX activity resulting in the need for regenerating AA, it is thought that concurrently an increase in various other components of the antioxidative defence system is needed so that the protective mechanisms of plants can increase as necessary. Peroxidase, CAT and APX appear to play an essential protective role in the scavenging process when coordinated with SOD activity. They scavenge H_2O_2 generated primary through SOD action [Chaitanya et al. 2002].

Many reports suggest that the extent of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of their antioxidant systems and the relationship between enhanced or constitutive antioxidant enzyme activities and an increased resistance to drought stress [El-Tayeb 2006, Liu et al. 2009, Basu et al. 2010, Kusvuran et al. 2016]. In this context, it is believed that a simultaneous increase in several components of the antioxida-

tive defense system would be necessary in order to obtain an increase in the plant protective mechanisms [Sanchez-Rodriguez et al. 2010].

Plant secondary metabolites are often referred to as compounds that have no fundamental role in the maintenance of life processes in the plants, but they are important for the plant to interact with its environment for adaptation and defense [Ramakrishna and Ravishankar 2011]. Accumulation of metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules [Bennett and Wallsgrove 1994]. Drought often causes oxidative stress and was reported to show increase in the amounts of flavonoids and phenolic acids in willow leaves. Ramakrishna and Ravishankar [2011] indicated that flavonoids have protective functions during drought stress. Therefore drought often causes oxidative stress and was reported to show increase in the amounts of flavonoids and phenolic acids in willow leaves. Several reports have indicated flavonoids are the group of secondary metabolites. The polyphenolic compounds, widely present in different plants and considered as an important factors of the overall antioxidant activity [Hertog et al. 1993, Ramakrishna and Ravishankar 2011]. Phenolic metabolites such as flavonoids, tannins, hydroxycinnamate esters and the structural polymer lignin, these compounds are often induced by stress and serve specific roles in plant protection, e.g., in pathogen defense or ultraviolet screening or as antioxidants, or antiherbivory or structural components of the cell wall [Hernandez et al. 2004].

The purpose of this study was to assess the physiological and biochemical response mechanisms adapted by two tomato genotypes which differing to tolerate drought and to assess whether a certain degree of drought stress could enhance the antioxidative enzyme activities, the total flavonoid, phenolic and proline contents of tolerant and sensitive tomato genotypes.

MATERIAL AND METHOD

Two tomato (*Solanum lycopersicum*) genotypes were used in this research: Tom-143 (drought-tolerant) and Tom-163 (drought-sensitive) [Dasgan et al. 2010]. Seeds were obtained from University of Cukurova, Department of Horticulture. Plants were grown in plastic pots (11 L) the containing a peat: perlite (2 : 1) ration in a greenhouse (temperature: 25°C ±2 and relative humidity: 55% ±5). Starting from 29 d after sowing, three watering treatments were applied: one well-watered treatment (100% of field capacity (FC): S1) and two water-stressed treatments (50 and 25% of FC: S2 and S3, respectively). The plants were subject to drought stress for 27 days. Control plants were grown under non-stress conditions for the same period of time.

Responses of the genotypes to drought were evaluated using some plant physiological (shoot fresh weights, leaf number, leaf area, relative water content) and biochemical parameters such as proline; total phenolic content (TPC), flavonoids, and chlorophyll content; lipid peroxide content (MDA); superoxide dismutase (SOD), catalase (CAT), ascorbat peroxidase (APX), and glutathione reductase antioxidative enzyme activities.

For total phenolic content (TPC), flavonoids and enzyme activity analyses were used leaf from the mid-top leaves of the plant. The total phenolic content was determined using a Folin-Ciocalteu reagent. The phenolic content of leaves and shoot was expressed in milligrams. Gallic acid was used as a standard [Singleton et al. 1999]. Flavonoid content was determined by colorimetric assay [Molina-Quijada et al. 2010, Medina-Juárez et al. 2012]. Total flavonoids were expressed on a fresh weight (fw) basis as milligrams of quercetin equivalents per gram. The proline was measured following the methods of Bates et al. [1973]. The proline was extracted from 100 mg dry weight (DW) of leaf samples with 2 mL of 40% methanol. Next, 1 mL of the extract was mixed with 1 mL of a mixture of glacial acetic acid and orthophosphoric acid (6 M) (3:2, v/v) and 25 mg ninhydrin. After 1 h incubation at 100°C, the tubes were cooled and 5 mL of toluene was added. The absorbance of the upper phase was spectrophotometrically measured at 528 nm.

Enzymes were extracted from 0.5 g of leaf tissue using a mortar and pestle, with 5 mL of extraction buffer containing 50 mM of potassium-phosphate buffer (pH 7.6) and 0.1 mM of disodium ethyl-

enediaminetetraacetate. The homogenate was centrifuged at 15 000 × g for 15 min, and the supernatant fraction was used to assay for the enzymes. All of the operations for the preparation of the enzyme extractions were performed at 4°C. The SOD was assayed according to Karanlik [2001], by monitoring the superoxide radical-induced nitro blue tetrazolium (NBT) reduction at 560 nm. The CAT activity was determined by monitoring the disappearance of HO. APX activity was determined by measuring the consumption of ascorbate from its the absorbance at 290 nm. One unit of APX activity was defined as the amount of enzyme required to consume 1 µmole of ascorbate min^{-1} [Cakmak and Marschner 1992]. The GR activity was determined by measuring the enzyme-dependent oxidation of NADPH from its the absorbance at 340 nm. One unit of GR activity was defined as the amount of enzyme that oxidized 1 µmole of NADPH min^{-1}.

The lipid peroxidation was measured as the amount of malondialdehyde (MDA) determined by the thiobarbituric acid (TBA) reaction [Heath and Packer 1968]. The MDA content was calculated according to the molar extinction coefficient of the MDA (155 mM^{-1} cm^{-1}).

RESULTS AND DISCUSSION

Present study investigated the morphological and biochemical performance of two tomato genotypes exposed to different levels drought stresses. Results showed that drought stress considerably reduced the growth of tomato genotypes in terms of fresh weight, shoot height, leaf number, and leaf area (tab. 1). Drought stress adversely affects the meristematic activity, cell elongation, results in premature abscission of leaves and roots, and reduces the photosynthetic activity and accumulation of dry matter [Latif et al. 2016]. The fresh weight was decreased by 49.9% under moderate stress (S2) in Tom-163. However, the decrease in fresh weight reached to 28% in Tom-143 and 63% in Tom-163 under S3 condition compared with control group. The shoot length of Tom-143 and Tom-163 were dramatically decreased depending on different drought stress levels. The shoot length of the tolerant Tom-143 genotype under S2 and S3 drought conditions were decreased by 9.8% and 11.76%, respectively. However, that of the sensitive Tom-163 genotype under these stress conditions was decreased by 31.8% and 60.4%, respectively. Photosynthesis and growth are the primary processes to be affected by drought [Sapeta et al. 2012]. The decrease may have been due to decline in net assimilation, brought about by decreased leaf water potential. Pugnaire et al. [1999] indicated that water stress reduces plant growth by reducing cell division and enlargement and causes a decline in transport to the root surface, which leads to a further decrease in plant growth. An early morphological response to drought stress is the avoidance mechanism through adjustment of plant growth rate such as a reduction in shoot height, basal diameter, and total fresh mass in the two Tom-143 and Tom-163 genotypes used in our experiment. The drought resulted in a reduction of total leaf area and leaf number in both genotypes at the end of the experiment. With drought stress leaf area decreased by 8.1–12.1% in Tom-143, however, this decreasing was determinated by 11.1–24.0% in Tom-163 under S2 and S3 treatment compared with their control groups, respectively. Development of optimal leaf area is important to photosynthesis and dry matter yield. Water deficit stress mostly reduced leaf growth and leaf area [Jaleel et al. 2009].

Drought stress defined that decrease of relative water content close stomata and after blocking of stomata will reduce photosynthesis rate. It is reported that high relative water content is a resistant mechanism to drought, and that high relative water content is the result of more osmotic regulation or less elasticity of tissue cell wall [Keyvan 2010]. The highest RWC (Relative Water Content) values were obtained in control groups (89–91%) (tab. 1). In tomato genotypes exposed to different levels of drought stress, RWC content decreased when compared to their controls. Under S2 and S3 stress conditions, the RWC decreased with the severity of drought stress. The decrease that was observed in the sensitive (Tom-163) genotype under drought stress was by 51% at S3 compared with that of S1 (control). Relative water content is considered a measure of plant water status, reflecting the metabolic activity in tis-

sues and used as a most meaningful index for dehydration tolerance. Anjum et al. [2011] indicated that RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures. RWC related to water uptake by the roots as well as water loss by transpiration. In this study, depending on decreasing relative water content could be caused reduction in leaf area around 24% in sensitive genotypes (Tom-163).

Plants tend to adapt to drought by accumulation of cyto-compatible organic osmolytes such as polyols, proline and betaines [Lakzayi et al. 2014]. The proline concentration in both of the tomato increased in water stress (tab. 2). After 27 days of water stress, the proline concentration of Tom-143 reached 2.05 and 3.17 µmol g^{-1} FW in the S2 and S3 treatments. However, under the same conditions, proline concentration of the Tom-163 genotype was 1.90 and 1.96 µmol g^{-1} FW, respectively. Drought increased proline content differently in sensitive and tolerant genotypes and greater proline accumulations in tolerant one were observed which correlates to drought level. Plants accumulate various soluble substances in their cytoplasm and organelles to obtain osmotic regulation during stress exposure. Many studies have proved a positive correlation between the stress tole-

Table 1. Changes in the morphological parameters of two tomato genotypes treated for different drought stress (S1: 100% of field capacity – Control; S2: 50% of field capacity – moderate stress; S3: 0% of field capacity – severe stress)

Genotype		Fresh weight (g·plant^{-1})	Shoot height (cm·plant^{-1})	Leaf number (number·plant^{-1})	Leaf area (cm^2·plant^{-1})	RWC (%)
Tom-143	S_1	37.00 ±5.29^{a}	34.00 ±2.00^{b}	9.33 ±1.53	447.03 ±8.49^{a}	91.00 ±2.00^{a}
	S_2	37.33 ±3.79^{a}	30.66 ±4.04^{b}	8.66 ±1.15	410.75 ±9.37^{b}	78.66 ±3.06^{b}
	S_3	26.33 ±4.93^{b}	30.00 ±3.46^{b}	8.33 ±0.58	392.95 ±5.77^{bc}	60.33 ±5.69^{c}
Tom-163	S_1	33.33 ±5.77^{ab}	34.00 ±4.00^{a}	10.66 ±1.15	417.55 ±4.60^{b}	89.33 ±2.52^{a}
	S_2	16.66 ±2.89^{c}	29.33 ±1.53^{b}	7.33 ±1.15	371.21 ±7.41^{c}	65.67 ±5.51^{c}
	S_3	12.33 ±2.52^{c}	17.00 ±1.00^{c}	6.33 ±1.53	217.27 ±4.37^{d}	43.66 ±6.66^{d}

* Results are means ±SD (n = 3). The different superscript letters indicate statistically significant differences by a Duncan's multiple range test ($P \leq 0.05$)

Table 2. Changes in the proline, chlorophyll, total phenolic, and total flavonoid contents of two tomato genotypes treated for different drought stress (S1: 100% of field capacity – Control; S2: 50% of field capacity – moderate stress; S3: 0% of field capacity – severe stress)

Genotype		Proline (µmol g^{-1} FW)	Chlorophyll (mg·FW^{-1})	Total phenolic contents (µg GAE·ml^{-1})	Total flavonoid content (mg QE·100 g^{-1})
Tom-143	S_1	1.41 ±0.14^{d}	59.40 ±4.77^{bc}	10.69 ±0.56^{d}	3.55 ±0.39^{e}
	S_2	2.05 ±0.08^{b}	62.30 ±3.61$^{a–c}$	17.24 ±1.65^{c}	9.24 ±0.51^{c}
	S_3	3.17 ±0.31^{a}	54.73 ±3.46^{c}	31.21 ±1.83^{a}	14.03 ±0.61^{a}
Tom-163	S_1	1.59 ±0.12^{cd}	70.24 ±4.36^{a}	9.15 ±0.58^{d}	4.14 ±0.32^{e}
	S_2	1.90 ±0.12^{bc}	65.35 ±5.02^{ab}	24.87 ±4.48^{b}	7.73 ±1.18^{d}
	S_3	1.96 ±0.09^{b}	39.57 ±3.10^{d}	22.78 ±2.11^{b}	11.18 ±0.53^{b}

* Results are means ±SD (n = 3). The different superscript letters indicate statistically significant differences by a Duncan's multiple range test ($P \leq 0.05$)

rance and the synthesis of organic substances like glycinebetaine and proline [Jia et al. 2015]. In this study, the proline content increased with different levels of water stresses. This increase was by 45–124% in the tolerant genotype (Tom-143), on the other hands, this change to 19–23% in the sensitive genotype (Tom-163) when compared to the control plants. These facts showed that proline is an effective organic substance, not only in functioning as an osmolyte, but also in the cellular stabilization [Kusvuran et al. 2013].

Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate [Anjum et al. 2011]. The chlorophyll contents in stress were reduced by increasing the drought level compared to their controls (tab. 2). The sensitive genotype Tom-163 in control has higher chlorophyll than the tolerant Tom-143. The sensitive Tom-163 showed significantly higher chlorophyll or in same significance level in control and moderate water stress (S2), however the comparison of stress with their control showed that the tolerant tomato's chlorophyll content was lesser affected by drought. After 27 days of exposure to drought, there was a decrease in the chlorophyll contents by 7% and 44% in the sensitive genotype; however, this decrease in the tolerant genotype was by 5–8%, respectively. Photo inhibition and the photo destruction of pigments may have contributed to such alterations [El-Tayeb 2006]. Drought stress caused a large decline in the chlorophyll the total chlorophyll content in sunflower varieties investigated [Manivannan et al. 2007]. The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species [Mafakheri et al. 2010]. Similarly, Ghorbanli et al. [2013] reported that chlorophyll a and b ratio reduced in resistant species of tomato against low water condition and this indicated that photosystem II protects the plant against low water stress.

The phenolic compounds in tomato genotypes were changed by water stress (tab. 2). The total phenolic contents of Tom-143 significantly increased under moderate (S2) and severe (S3) water stress condition when compared with the control (224% and 265%, respectively). Similarly, total flavonoid content increased depending on water stress levels. In this study, total flavonoid content was determined to be 6.37 mgQE/100g (17% increase) and 9.40 mgQE/100g (73% increase) under S2 and S3 water stress conditions, respectively. On the contrary, in Tom-163 total flavonoid content decreased (4.9 mgQE/100g – 19% decrease) under S3 treatment (tab. 2). Plant secondary metabolites are often referred to as compounds that have no fundamental role in the maintenance of life processes in the plants, but they are important for the plant to interact with its environment for adaptation and defense [Ramakrishna and Ravishankar 2011]. Mansori et al. [2015] reported that polyphenols represent a large family of plant secondary metabolites and these may act as antioxidants to protect the plant against oxidative stress.

MDA content was measured in the leaves as an indicator of oxidative damage in plants under drought stress (tab. 3). The results showed that MDA increased significantly under water stress and reached highest levels (3.46 and 5.92 μmol g^{-1} FW) under S3 treatment in Tom-143 and Tom-163, respectively. This chance was more clearly observed due to the 733.8% increase in Tom-163 when compared to the control plants. These free radicals lead to irreversible damage to in lipids and proteins. Lipid peroxidation destroys the integrity of the cell membranes, and eventually, cell death occurs [Dolatabadian et al. 2008, Kusvuran et al. 2013]. The lipid peroxidation increase is due to compounds such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) in chloroplasts. In this study, lipid peroxidation of both genotypes increased with drought stress. However, this reduction was significant in the sensitive genotype (Tom-163) in different drought levels compared to the tolerant genotype (Tom-143). In a previous study [Rosales et al. 2012, Li et al. 2013, Mansori et al. 2015], the investigators showed that the MDA levels increased, especially in the susceptible phenotypes, depending on drought stress, and this increase was related with ROS formation. These results may be imputed to varieties in their genotypic ability to scavenge ROS and/or to be protected against their oxidative properties.

Table 3. Changes in the SOD, CAT, APX, GR enzyme activities and MDA content of two tomato genotypes treated for different drought stress (S1: 100% of field capacity – Control; S2: 50% of field capacity – moderate stress; S3: 0% of field capacity – severe stress)

Genotype		SOD (U min^{-1} mg^{-1} FW)	CAT (μmol min^{-1} mg^{-1} FW)	APX (μmol min^{-1} mg^{-1} FW)	GR (μmol min^{-1} mg^{-1} FW)	MDA (μmol g^{-1} FW)
Tom-143	S_1	24.19 ±11.35^{e}	34.56 ±6.28^{c}	120.39 ±2.80^{e}	13.75 ±0.36^{d}	0.76 ±0.06^{e}
	S_2	64.28 ±6.49^{c}	105.21 ±17.32^{b}	162.23 ±2.42^{c}	31.81 ±5.23^{bc}	2.24 ±0.12
	S_3	132.85 ±3.54^{a}	253.82 ±21.67^{a}	202.17 ±9.99^{a}	51.67 ±2.44^{a}	3.46 ±0.48^{c}
Tom-163	S_1	39.62 ±7.57^{d}	51.64 ±5.52^{c}	193.23 ±2.92^{d}	17.47 ±1.12^{d}	0.71 ±0.07^{e}
	S_2	47.21 ±4.40^{d}	102.56 ±20.05^{b}	165.08 ±4.01^{c}	33.79 ±4.18^{b}	4.33 ±0.22^{b}
	S_3	87.43 ±12.36^{b}	103.14 ±15.95^{b}	180.85 ±6.96^{b}	25.84 ±4.62^{c}	5.92 ±0.98^{a}

* Results are means ±SD (n = 3). The different superscript letters indicate statistically significant differences by a Duncan's multiple range test ($P \leq 0.05$)

The drought stress activated the antioxidant system in tomato genotypes. In the S3 treatment, the SOD activity reached 132.85 U min^{-1} mg^{-1} FW in Tom-143; however, it only reached 87.43 U min^{-1} mg^{-1} FW in the sensitive genotype (tab. 3). The SOD activity increase was higher in the tolerant genotypes (449%) compared to the sensitive genotypes (121%) under S3 treatment. Similar trends were observed for CAT activity which increased during the S2 treatment, reaching maximal levels during the S3 treatment (tab. 3). However, CAT activity of the Tom-143 was significantly higher (195–634%) than Tom-163 (98–99%) during both the S2 and S3 applications. Superoxide radicals that emerge as a result of stress in the plant tissues are transformed into hydrogen peroxide by the SOD enzyme. The accumulation of H_2O_2, which results from the change reaction of the SOD enzyme and is a powerful oxidant, is prevented by the ascorbate–glutathione cycle. SOD and CAT, by combining their actions, can prevent or decrease the formation of this oxide [Kusvuran et al. 2016]. Our results showed that both genotypes induced SOD and CAT activities upon drought, consistent with the increment in peroxidation levels. At the same time, these enzymatic activities were higher in the drought tolerant genotype than in the drought sensitive genotype.

The results showed that APX and GR activities increased under stress conditions compared to their controls (tab. 3). The highest APX and GR activities were determined to be 202.17 and 51.67 μmol min^{-1} mg^{-1} FW, respectively, in Tom-143 under S3 treatment. The APX uses ascorbate as an electron donor to reduce H2O2 to water. The main function of APX is the removal of toxic H2O2 and thereby protecting plants during oxidative stress. GR activity increased during severe water stress. GR catalyses the NADP-dependent reduction of GSSG to generate reduced glutathione which plays an important role during the removal of dioxygen under stress conditions. The regeneration of GSH from oxidized glutathione (GSSG) by GR is very important since only the reduced form of GSH can take part in the removal of active oxygen species [Slabbert and Krüger 2014]. Increased SOD, CAT, APX, and GR activities in tolerant plants could reduce the amount of damage caused by various stress conditions [Dawood et al. 2014]. Hence, it is proposed that these anitoxidative enzymes may play important roles in the rapid defence responses of plant cells against oxidative stress. Reactive oxygen species (ROS) are generated in plant cells by normal cellular metabolism or due to unfavorable environmental conditions such as drought, salinity, heavy metals, herbicides, nutrient deficiency, or radiation. Their productions are controlled by various enzymatic and non-enzymatic antioxidant defense systems. Enzymatic antioxidant defense systems, including CAT, APX, POX, SOD, MDHAR, DHAR and GR and non-enzymatic antioxidant de-

fense systems, including ascorbate, glutathione, carotenoids, phenolic compounds, proline, glycine betain, sugar, and polyamines [Sen 2012].

Conclusively, we found that *Solanum lycopersicum* genotypes could differently enhance their ability to struggle the drought. Our results showed that drought tolerant Tom-143 challenged more successfully than sensitive Tom-163 due to more effectively up-regulating antioxidative systems and making osmotic adjustments in response to drought stress. It is possible that proline, secondary metabolite accumulations like total phenolics and flavonoids and antioxidative enzyme activities could be used as the effective mechanisms for drought tolerance in Tom-143.

REFERENCES

Anjum, S.A., Xie, X.Y., Wang, L.C., Saleem, M.F., Man, C., Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agri. Res., 6(9), 2026–2032.

Asada, K. (1999). The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Ann. Rev. Plant Biol., 50(1), 601–639.

Basu, S., Roychoudhury, A., Paromita Saha, P., Sengupta, D.N. (2010). Differential antioxidative responses of indica rice cultivars to drought stress. Plant Growth Reg., 60, 51–59.

Bates, L.S., Waldren, R.P., Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant Soil, 39(1), 205–207.

Bennett, R.N., Wallsgrove, R.M. (1994). Secondary metabolites in plant defence mechanisms. New Phytol., 127, 617–33.

Chaitanya, K.V., Sundar, D., Masilamani, S., Reddy, A.R. (2002). Variation in heat stress-induced antioxidant enzyme activities among three mulberry cultivars. Plant Growth Reg., 36(2), 175–180.

Dawood, M.G., Taie, H.A.A., Nassar, R.M.A., Abdelhamid, M.T., Schmidhalter, U. (2014). The changes induced in the physiological, biochemical and anatomical characteristics of Vicia faba by the exogenous application of proline under seawater stress. South Afr. J. Bot., 93, 54–63.

Dixit, V., Pandey, V., Shyam, R. (2001). Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). J. Exp. Bot., 52, 1101–1109.

Dolatabadian, A., Sanavy, S.A.M.M., Chashmi, N.A. (2008). The effects of foliar application of ascorbic acid (vitamin C) on antioxidant enzymes activities, lipid peroxidation and proline accumulation of canola (*Brassica napus* L.) under conditions of salt stress. J. Agron. Crop Sci., 194(3), 206–213.

El-Tayeb, M.A. (2006). Differential response of two *Vicia faba* cultivars to drought: growth, pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity. Acta Agron. Hung., 54, 25–37.

Ghorbanli, M., Gafarabad, M., Amirkian, T.A.N.N.A.Z., Mamaghani, B.A. (2013). Investigation of proline, total protein, chlorophyll, ascorbate and dehydroascorbate changes under drought stress in Akria and Mobil tomato cultivars. Iran J. Plant Physiol., 3(2), 651–658.

Heath, R.L., Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys., 125(1), 189–198.

Hernández, I., Alegre, L., Munné-Bosch, S. (2004). Drought-induced changes in flavonoids and other low molecular weight antioxidants in Cistus clusii grown under Mediterranean field conditions. Tree Physiol., 24(11), 1303–1311.

Hertog, M.G.L., Hollman, P.C.H., Katan, M.B., Kromhout, D. (1993). Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. Nutr. Cancer, 20, 21–29.

Jaleel, C.A., Riadh, K., Gopi, R., Manivannan, P., Inès, J., Al-Juburi, H.J., Panneerselvam, R., (2009). Antioxidant defense responses: physiological plasticity in higher plants under abiotic constraints. Acta Physiol. Plant., 31(3), 427–436.

Jia, X., Sun, C., Li, G., Li, G., Chen, G. (2015). Effects of progressive drought stress on the physiology, antioxidative enzymes and secondary metabolites of Radix Astragali. Acta Physiol. Plant., 37(12), 1–14.

Karanlik S. (2001). Resistance to salinity in different wheat genotypes and physiological mechanisms involved in salt resistance. Ph.D. Thesis. Institute of Natural and Applied Sciences, University of Cukurova, Adana, 122 pp (in Turkish).

Keyvan, S. (2010). The effects of drought stress on yield, relative water content, proline, soluble carbohydrates

and chlorophyll of bread wheat cultivars. J. Anim. Plant Sci, 8(3), 1051–1060.

Kusvuran, S., Ellialtioglu, S., Polat, Z. (2013). Antioxidative enzyme activity, lipid peroxidation, and proline accumulation in the callus tissues of salt and drought tolerant and sensitive pumpkin genotypes under chilling stress. Hort. Envir. Biotech., 54(4), 319–325.

Kusvuran, S., Kıran, S., Ellialtıoğlu, Ş.Ş. (2016). Antioxidant enzyme activities and abiotic stress tolerance relationship in vegetable crops. In: Abiotic and biotic stress in plants – recent advances and future perspectives, Shanker, A.K., Shanker, Ch. (eds). InTech, pp. 481–506.

Lakzayi, M., Sabbagh, E., Rigi, K., Keshtehgar, A. (2014). Effect of salicylic acid on activities of antioxidant enzymes, flowering and fruit yield and the role on reduce of drought stress. Int. J. Farming All. Sci., 3(9), 980–987.

Latif, F., Ullah, F., Mehmood, S., Khattak, A., Khan, A.U., Khan, S., Husain, I. (2016). Effects of salicylic acid on growth and accumulation of phenolics in Zea mays L. under drought stress. Acta Agric. Scand. Sec. B Soil Plant Sci., 66(4), 325–332.

Li, Z., Peng, Y., Ma, X. (2013). Different response on drought tolerance and post-drought recovery between the small-leafed and the large-leafed white clover (*Trifolium repens* L.) associated with antioxidative enzyme protection and lignin metabolism. Acta Physiol. Plant., 35(1), 213–222.

Liu, Z.J., Zhang, X.L., Bai, J.G., Suo, B.X., Xu, P.L., Wang, L. (2009). Exogenous paraquat changes antioxidant enzyme activities and lipid peroxidation in drought-stressed cucumber leaves. Sci. Hort., 121, 138–143.

Lopez‐Huertas, E., Charlton, W.L., Johnson, B., Graham, I.A., Baker, A. (2000). Stress induces peroxisome biogenesis genes. The EMBO J., 19(24), 6770–6777.

Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P.C., Sohrabi, Y. (2010). Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Austral. J. Crop Sci., 4(8), 580.

Mansori, M., Chernane, H., Latique, S., Benaliat, A., Hsissou, D., El Kaoua, M. (2015). Seaweed extract effect on water deficit and antioxidative mechanisms in bean plants (*Phaseolus vulgaris* L.). J. Appl. Phycol., 27(4), 1689–1698.

Medina-Juárez, L.A., Molina-Quijada, D.M., Del Toro-Sánchez, C.L., González-Aguilar, G.A., Gámez-Meza, N. (2012). Antioxidant activity of peppers (*Capsicum annuum* L.) extracts and characterization of their phenolic constituents. Interciencia, 37(8), 588–593.

Mittiova, V., Tal, M., Volokita, M., Guy, M. (2002). Salt stress induces up-regulation of an efficient chloroplast antioxidant system in the salt-tolerant wild tomato species *Lycopersicon pennelii* but not in the cultivated species. Physiol. Plant., 115(3), 393–400.

Molina-Quijada, D.M.A., Medina-Juárez, L.A., González-Aguilar, G.A, Robles-Sánchez, R.M. and Gámez-Meza, N. (2010). Phenolic compounds and antioxidant activity of table grape (Vitis vinifera L.) skin from northwest Mexico. Cien. Tec. Aliment., 8(1), 57–63.

Pugnaire, F.I., Serrano, L., Pardos, J. (1999). Constraints by water stress on plant growth. Handb. Plant Crop Stress, 2, 271–283.

Ramakrishna, A., Ravishankar, G.A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav., 6, 1720–1731.

Rosales, M.A., Ocampo, E., Rodríguez-Valentín, R., Olvera-Carrillo, Y., Acosta-Gallegos, J., Covarrubias, A.A. (2012). Physiological analysis of common bean (*Phaseolus vulgaris* L.) cultivars uncovers characteristics related to terminal drought resistance. Plant Physiol. Biochem., 56, 24–34.

Sánchez-Rodríguez, E., Rubio-Wilhelmi, M., Cervilla, L.M., Blasco, B., Rios, J.J., Rosales, A., Romero, L., Ruiz, J.M. (2010). Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. Plant Sci., 178(1), 30–40.

Sapeta, H., Costa, J.M., Lourenco, T., Maroco, J., Van der Linde, P., Oliveira, M.M. (2013). Drought stress response in Jatropha curcas: growth and physiology. Environ. Exp. Bot., 85, 76–84.

Sen, A. (2012). Oxidative stress studies in plant tissue culture. Antioxidant Enzym., 3, 59–88.

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol., 299, 152–178.

Slabbert, M.M., Krüger, G.H.J. (2014). Antioxidant enzyme activity, proline accumulation, leaf area and cell membrane stability in water stressed Amaranthus leaves. South Afr. J. Bot., 95, 123–128.

Yuan, G.F., Jia, C.G., Li, Z., Sun, B., Zhang, L.P., Liu, N., Wang, Q.M. (2010). Effect of brassinosteroids on drought resistance and abscisic acid concentration in tomato under water stress. Sci. Hort., 126(2), 103–108.