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INVESTIGATION OF DROUGHT STRESS IN PEPINO (*SOLANUM MURICATUM* AIT. CV. MISKI) LEAVES

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Abstract

In this study, the effects of drought stress on pepino seedlings (*Solanum muricatum* cv. Miski) under natural greenhouse conditions were examined. The control plants were watered at field capacity, and the stress group was not watered. Samples were collected on the 6th, 12th, 24th and 36th days from the leaves of *S. muricatum* seedlings that, were exposed to drought stress. The relative water content, the total phenolic compounds, and the malondialdehyde, total photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids), and proline contents in these samples were determined. Depending on drought stress, the relative water content, the chlorophyll a, chlorophyll b and carotenoid contents, and the total chlorophylls were found to be lower in the stress group compared with the control group. In contrast, the total phenolic compounds (24th and 36th days) and the proline (12th, 24th and 36th days) levels increased significantly compared with the control group. In addition, a significant increase in the malondialdehyde contents was obtained on the 36th day in the stress group compared with the control group. Such studies may be important for evaluation of metabolic changes in pepino under the drought stress.

Key words: Drought stress, Malondialdehyde, Phenolic compounds, Photosynthetic pigments, Proline, *Solanum muricatum* Ait.

Introduction

Solanum muricatum Aiton. is a member of the Solanaceae family and as commonly known as pepino (Anon., 1989; Anon., 1994). The green-striped unripe fruits taste like cucumber, whereas the ripe fruits taste like melon-mango and are rich in potassium (Francke, 2010). In addition, pepino is also rich in vitamin A and C (Anon., 1989; Anon., 1994; Rodriguez-Burruezo *et al.,* 2011). The pepino fruit can be used fresh, similar to a cucumber, in salads (Prohens *et al.,* 2002). It also exhibits several medical properties, such as its hypotension effect, diuretic function and antitumor activator (Redgwell & Turner, 1986; Ren & Tang, 1999). Hsu *et al.* (2011) determined that the aqueous extracts of pepino fruits may reduce the progression of diabetes due to the antioxidative, anti-inflammatory and antiglycative effects. The ethyl acetate extract of the pepino fruit has a very remarkable radical scavenger property, that this extract contains phenols and flavonoids in important ratios, and that the radical scavenging property of this extract is most likely caused by its polyphenol content (Sudha *et al.,* 2011).

One of the abiotic stresses that restrict plant production is drought stress, and this stress decreases the product yield (Boyer, 1982; Jaleel *et al.,* 2009). Water is necessary for the survival of the biochemical reactions within the plant cells to ensure the growth and development of the plant (Nobel, 1999; Yoo *et al.,* 2009). In response to the drought stress, plants undergo important morphological and metabolic changes, including insufficient growth, early ripening, decrease or increase in the root height, increase in the root-stem ratio, decrease in the total leaf area, and total leaf mass, and the rolling of leaves (Fischer & Wood, 1979; Karamanos & Papatheohari, 1999; Cattivelli *et al.,* 2008; Jaleel *et al.,* 2009).

A number of studies, have stated that, depending on the drought stress, the relative water content and the photosynthetic pigments decrease, the lipid peroxidation increases, and prolines accumulate (Fu & Huang, 2001; Türkan *et al.,* 2005; Kocsy *et al.,* 2005; Yin *et al.,* 2005; Çamoğlu *et al.,* 2011). Some studies also showed that, depending on the environmental stresses, the phenylpropanoids accumulate and that these play a regulatory role in several metabolic processes (Dixon & Paiva, 1995; Solecka, 1997; Janas *et al.,* 2000; Wrobel *et al.,* 2005).

To date, the effect of fertilization with potassium on the ripening of the fruit and the macro nutrient contents in the fruit of *S. muricatum* (Francke, 2010), the fruit yield and quality in different months (Çavuşoğlu *et al.,* 2009), and the effects of salinity stress and CO_2 on the growth and yield of the fruit (Chen *et al.,* 1999) have been researched. However, there were no reports on the physiological changes induced in *S. muricatum* by drought stress. Determination of physiological changes depending on drought stress in pepino is important in explaining some of the tolerance mechanisms, and create strategies for the future. Therefore, the aim of this study was to investigate the relative water content (RWC), the total phenolic compounds, the malondialdehyde (MDA), proline and total pigment (chlorophyll a, chlorophyll b and carotenoids) contents in the Miski cultivar of *S. muricatum* (pepino) exposed to drought stress.

Materials and Methods

Plant materials and stress treatments: In this study, the Miski cultivar of the pepino plant was used. Pepino seedlings were placed in equally sized plastic pots that contained a pearlite-peat (2:1) mixture. The study was conducted in a greenhouse with a nighttime temperature of 7°C and a daytime temperature of 29°C, a daytime humidity of 44% and a nighttime humidity of 72%, and the natural photoperiod conditions. After the seedlings were placed in the pots, they were acclimated for 15 days to their environment. The seedlings in the pots were then divided into two groups the control group and the stress group. The control group was watered once every two days at field

capacity, and the stress group, which was exposed to drought stress, and was not watered for 36 days. During these trials, samples were collected from the plant leaves on the 6th, 12th, 24th, and 36th days. The RWC, the chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids contents, the total phenolic compounds, and the MDA and proline amounts in the sampled plant leaves were analyzed.

Determining the relative water content: After the fresh weights of the leaf samples collected from the pepino plant on the 6th, 12th, 24th, and 36th days of the stress treatment were determined, the samples were induced to become turgescent by being maintained in water for four hours. After the turgescence weights of the leaves were weighed, the samples were maintained in an oven at 65°C for 48 hours to determine the dry weights. The percent RWCs were calculated using formula in the literature (Barr & Weatherley, 1962; Sairam *et al.,* 2002). The analyses were repeated thrice.

Determining the pigments: The method developed by De Kok & Graham (1989) was used in the extraction and the purification of the pigments. The absorbance values of the samples' were measured at 662, 645 and 470 nm according to the method described by Lichtenthaler & Wellburn (1983). The analyses were repeated thrice.

Determining the total phenolic compounds: Total phenolic compounds of samples were determined according to methods of Slinkard & Singleton (1977) and Chandler & Dodds (1983). The analyses were repeated thrice.

Determining the MDA contents: MDA contents were determined according to method of Heath & Packer (1968). The analyses were repeated thrice.

Proline analysis: This analysis was performed according to the method developed by Bates *et al*. (1973). The analyses were repeated thrice.

Data analysis: All of the analyses in this study were repeated thrice. The statistical evaluations of the obtained results were conducted using the SPSS 15.0 program. To determine the differences between the means, Duncan and *t*-tests were used. In the analyses, differences with $p<0.05$ were considered to be significant.

Results

Change in the RWCs: The RWC of the pepino plant within the control group which was not exposed to drought stress, was unchanged on the 6th, 12th, 24th, and 36th days ($p>0.05$) (Fig. 1). In contrast, the RWCs in the stress groups decreased throughout the trial. This decrease was significant up to the 24th day ($p<0.05$), and not significant on the 24th and 36th days ($p>0.05$) (Fig. 1). In the 6th day, the RWCs of the stress group decreased compared to the control group, and this difference was not significant ($p>0.05$). However, in the 12th, 24th, and 36th days, the RWCs of the plants in the stress group were significantly lower compared to the control group ($p<0.05$). The changes in the RWCs were found to be 49.21% and 48.69% in the control and the stress groups on the 6th day, 48.42%, and 31.02% on the 12th day, 47.34%, and 22.61% on the 24th day, and 47.56%, and 19.13% on the 36th day, respectively (Fig. 1).

Change in the pigments: No change was found in the Chl a contents of the control groups of pepino plants on the 6th, 12th, 24th, and 36th days ($p>0.05$) (Fig. 2). The Chl a levels in the stress group decreased as of the 12th day ($p<0.05$) (Fig. 2). In the stress group, the Chl a values were found to be 0.78 mg g^{-1} on the 12th day, 0.48 mg g^{-1} on the 24th day and 0.21 mg g^{-1} on the 36th day. The analysis of the changes in the Chl a levels of the control and stress groups revealed, a significant decrease in the stress group compared to the control on the 6th, 12th, 24th, and 36th days ($p<0.05$) (Fig. 2).

In the control groups of the pepino plants exposed to the drought stress, no significant change in the Chl b content was detected on the 6th, 12th, 24th, and 36th days (Fig. 3). The Chl b values in the stress group were found to have decreased as of the 12th day. It was found that the Chl b levels exhibited significant differences between the control and the stress groups ($p<0.05$) (Fig. 3). The Chl b contents in the control and the stress groups were 1.01 mg g^{-1} and 0.81 mg g^{-1} on the 6th day, 1.12 mg g^{-1} and 0.81 mg g^{-1} on the 12th day, 1.14 mg g^{-1} and 0.76 mg g^{-1} on the 24th day, and 1.24 mg g^{-1} and 0.40 mg g^{-1} on the 36th day, respectively (Fig. 3).

In the stress group the carotenoid contents were found to have decreased significantly compared with the control ($p < 0.05$) (Fig. 4). The carotenoid contents in the control group were not significantly changed on the 6th, 12th, 24th, and 36th days ($p>0.05$). In the stress group, the carotenoid contents were higher on the 6th day (0.44 mg g^{-1}) and lower on the 36th day (0.09 mg g^{-1}) (Fig. 4).

The total chlorophyll levels in the leaves of the pepino plant were found to have decreased significantly in the stress group at all the durations tested ($p<0.05$) (Fig. 5). It was also determined that the total chlorophyll contents in the control group did not change throughout the trials ($p>0.05$). In contrast, the total chlorophyll contents in the stress group decreased ($p<0.05$) (Fig. 5). The total chlorophyll contents in the control and the stress groups were found to be 2.51 mg g^{-1} and 1.73 mg g^{-1} on the 6th day, 2.50 mg g^{-1} and 1.60 mg g^{-1} on the 12th day, 2.45 mg g^{-1} and 1.25 mg g^{-1} on the 24th day, and 2.56 mg g^{-1} and 0.61 mg g^{-1} on the 36th day, respectively (Fig. 5).

Change in the total phenolic compounds: In the control groups, the total phenolic compounds were higher on the 24th day and exhibited similar values on the 6th, 12th, 36th days (Fig. 6). In contrast, in the stress groups, the total phenolic compounds were found to be higher on the 24th day and to exhibit the lowest value on the 6th day, similarly to the control group (Fig. 6). The analysis of the total phenolics in the stress and the control groups revealed that the changes in the 6th and 12th days were not significant ($p>0.05$) and that the changes in the 24th and 36th days were significant ($p<0.05$). The total phenolics in the control and the stress groups were determined to be 0.74 µg mg^{-1} and 0.94 µg mg^{-1} on the 24th day, and 0.67 µg mg^{-1} and 0.87 µg mg^{-1} on the 36th day, respectively (Fig. 6).

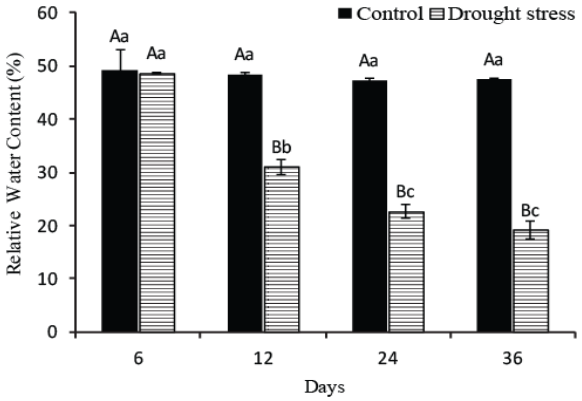

Fig. 1. The change of RWCs in the pepino leaves under the drought stress
Different letters are significant in terms of statistics (p<0.05). While the small letters indicates the comparison between the averages according to Duncan Comparison Test, the capital letters indicates the comparison between the averages according to *t*-test (confidence limit 95 %).

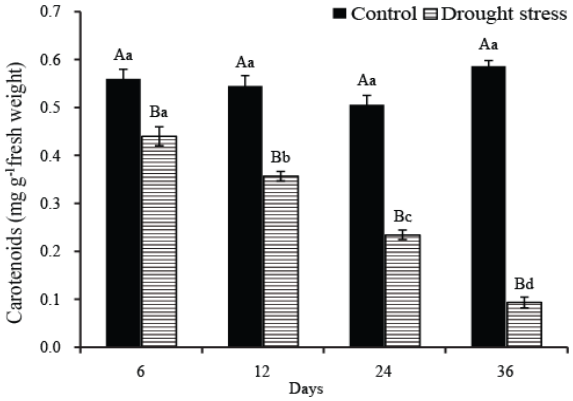

Fig. 4. The change of carotenoids in the pepino leaves under the drought stress (mg g^{-1} fresh weight)
Different letters are significant in terms of statistics (p<0.05). While the small letters indicates the comparison between the averages according to Duncan Comparison Test, the capital letters indicates the comparison between the averages according to *t*-test (confidence limit 95 %).

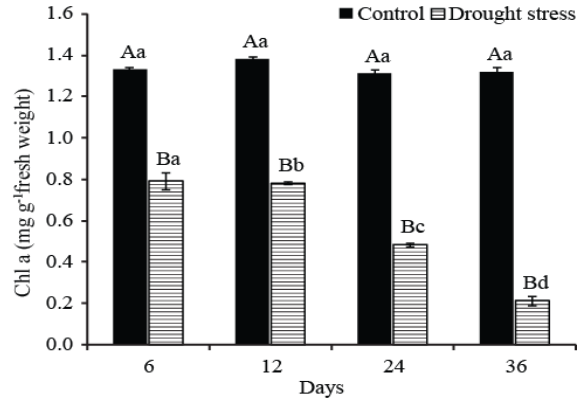

Fig. 2. The change of Chl a contents in the pepino leaves under the drought stress (mg g^{-1} fresh weight)
Different letters are significant in terms of statistics (p<0.05). While the small letters indicates the comparison between the averages according to Duncan Comparison Test, the capital letters indicates the comparison between the averages according to *t*-test (confidence limit 95 %).

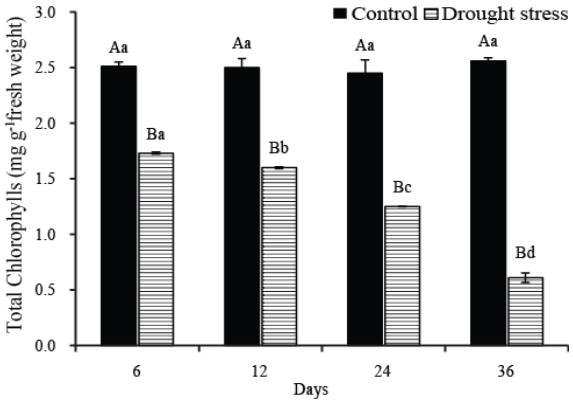

Fig. 5. The change of total chlorophyll contents in the pepino leaves under the drought stress (mg g^{-1} fresh weight)
Different letters are significant in terms of statistics (p<0.05). While the small letters indicates the comparison between the averages according to Duncan Comparison Test, the capital letters indicates the comparison between the averages according to *t*-test (confidence limit 95 %).

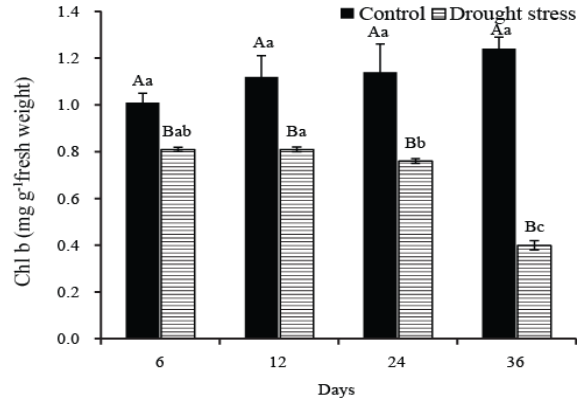

Fig. 3. The change of Chl b contents in the pepino leaves under the drought stress (mg g^{-1} fresh weight)
Different letters are significant in terms of statistics (p<0.05). While the small letters indicates the comparison between the averages according to Duncan Comparison Test, the capital letters indicates the comparison between the averages according to *t*-test (confidence limit 95 %).

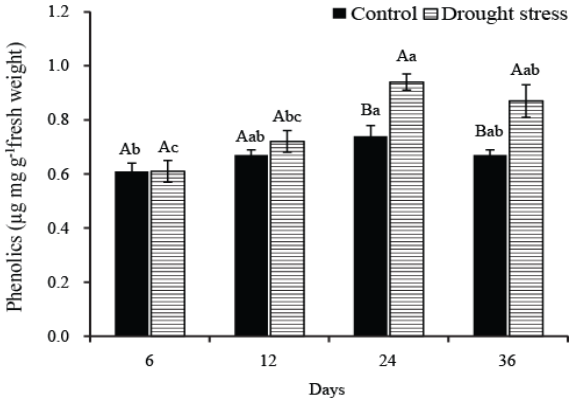

Fig. 6. The change of total phenolics in the pepino leaves under the drought stress (µg mg^{-1} fresh weight)
Different letters are significant in terms of statistics (p<0.05). While the small letters indicates the comparison between the averages according to Duncan Comparison Test, the capital letters indicates the comparison between the averages according to *t*-test (confidence limit 95 %).

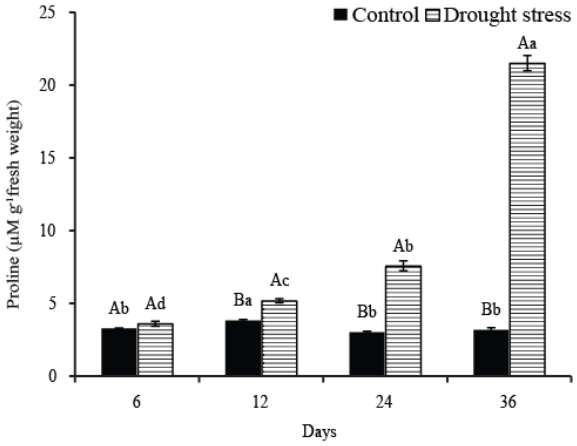

Fig. 7. The changes in proline in the pepino leaves under the drought stress (µM g^{-1} fresh weight)
Different letters are significant in terms of statistics ($p<0.05$). While the small letters indicates the comparison between the averages according to Duncan Comparison Test, the capital letters indicates the comparison between the averages according to *t* test (confidence limit 95 %).

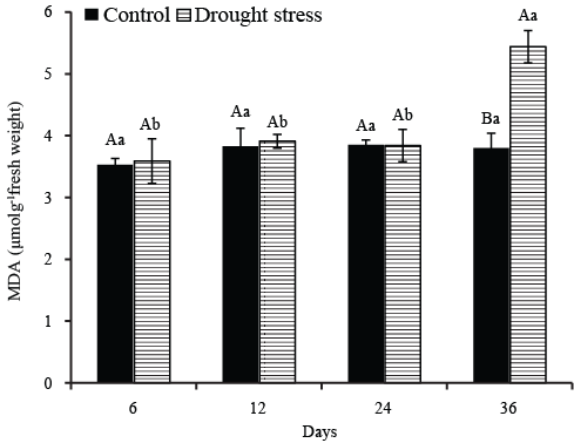

Fig. 8. The changes in MDA in the pepino leaves under the drought stress (µmol g^{-1} fresh weight)
Different letters are significant in terms of statistics ($p<0.05$). While the small letters indicates the comparison between the averages according to Duncan Comparison Test, the capital letters indicates the comparison between the averages according to *t* test (confidence limit 95 %).

Proline changes: The examination of the proline amounts in the pepino plants, revealed that there were no changes in the control group. However, in the stress group, the changes in the proline amounts between the 6^{th}, 12^{th}, 24^{th}, and 36^{th} days were determined to be significant ($p<0.05$) (Fig. 7). The differences in the proline amounts between the control and the stress groups were found to be not significant on the 6^{th} day ($p>0.05$) and significant on the 12^{th}, 24^{th}, and 36^{th} days ($p<0.05$). The highest proline content was 21.50 µM g^{-1} (in the stress group on the 36^{th} day). However, the proline content in the stress group on the other days was determined to be 3.60 µM g^{-1} on the 6^{th} day, 5.18 µM g^{-1} on the 12^{th} day, and 7.57 µM g^{-1} on the 24^{th} day (Fig. 7).

MDA changes: The changes in the MDA contents in the stress group were found to be insignificant on the 6^{th}, 12^{th} and 24^{th} days ($p>0.05$) but significant on the 36^{th} day ($p<0.05$) (Fig. 8). In the control groups, the MDA contents were unchanged. The differences in the MDA contents between the control and the stress groups were not significant ($p>0.05$) on the 6^{th}, 12^{th}, 24^{th} days, and significant on the 36^{th} day ($p<0.05$). In the control group, the MDA contents were 3.53 µmol g^{-1} on the 6^{th} day, 3.83 µmol g^{-1} on the 12^{th} day, 3.85 µmol g^{-1} on the 24^{th} day, and 3.80 µmol g^{-1} on the 36^{th} day. In the stress group, the MDA contents were found to be 3.59 µmol g^{-1} on the 6^{th} day, 3.91 µmol g^{-1} on the 12^{th} day, 3.84 µmol g^{-1} on the 24^{th} day, and 5.44 µmol g^{-1} on the 36^{th} day (Fig. 8).

Discussion

RWC is indicator of plant water status (Schonfeld *et al.,* 1988). Terzi & Kadioğlu (2006), studied the relationship between drought stress tolerance and the antioxidant enzyme system, during rolling in the leaf, root and petiole of *Ctenanthe setosa*, and found that the RWC decreased depending on the drought. Kalefetoğlu & Ekmekçi (2009), examined the characterization of the resistance against drought stress in chickpea breeds and lines and found that in response to drought stress, the RWCs in the genotypes decreased compared to the control. David (2002) stated that the reduction of leaf RWC was caused reduction in photosynthesis, transpiration and stomatal conductance. In the present study we conducted that RWCs of pepino plant decreased depending on the drought stress (Fig. 1), and this finding is in agreement with those of the abovementioned studies. The reduction in RWC of pepino, as indicated above study (David, 2002) may be cause to decreasing in photosynthesis.

One of the parameters that are affected in response to drought stress is the photosynthetic pigments. Çamoğlu *et al.,* (2011) investigated the effect of water stress on the water consumption and other, physiological and biochemical parameters in sweet corn and found that, in parallel with the increase in the water stress, the chlorophyll contents markedly decreased. In *Hordeum vulgare* L. breeds, these researchers discovered that, depending on the water stress, the total chlorophyll ratios decreased in both breeds, although the Chl a and Chl b content changes differed depending on the breeds (Anjum *et al.,* 2003). Manivannan *et al*. (2007) researched the effects of drought stress on the growth, biochemical changes and proline metabolism of different varieties of the *Helianthus annuus* L. In their study, these researchers, found a decrease in the Chl a, Chl b and total chlorophyll levels. According to our findings, the Chl a, Chl b, total chlorophylls and carotenoid contents decreased depending on the exposure time of the pepino seedlings to drought stress (Figs. 2, 3, 4 and 5). Chlorophyll content is one of the significant factors affecting photosynthetic capacity (Arjenaki *et al.,* 2012). The decrease in chlorophyll content under the drought stress is mainly the result of pigment photooxidation and chlorophyll degradation (Anjum *et al.,* 2011).

Water deficit may cause oxidative stress in plants. Reactive oxygen species and free radicals generated by oxidative stress can be inactivated by number of mechanisms in plants. One of the defense mechanisms employs small molecule compounds, such as glutathione, ascorbate, carotenoids, flavonoids, tocopherols, phenols, proline etc. (Weidner *et al.,* 2009). In the present study, it was found that the total phenolic compounds increase in the stress groups compared to the control in response to drought stress (Fig. 6). Oh *et al.* (2010) determined that antioxidant capacity and total phenolic compounds in lettuce increased by the graded lack of water. Mehrjerdi *et al.* (2013) investigated the phenolic compounds, radical cleaning activities, and photosynthetic characteristics in response to drought stress in different chickpea genotypes under hydroponic conditions and discovered that the total phenols, photosynthetic ratio, transpiration, Chl a, Chl b, water usage efficiency, and membrane determination index decreased with an increase in the drought stress. Ahmed *et al.* (2012) found that the total phenolics in *in vitro* cultures of the garden peppergrass increase in response to drought and salinity stress compared to the control. The analysis of the abovementioned studies reveals that phenolic compounds increase under varying stress conditions.

Proline is one of the most common osmolytes accumulated during drought stress (Mafakheri *et al.,* 2010). Additionally proline can play as an electron receptor preventing photosystems damages in dealing with ROS function (Ghorbanli *et al.,* 2013). Zou *et al.* (2012) detected that the proline contents increase in rice in response to drought and salinity stresses. Deng *et al.* (2012) investigated the drought tolerance of intergeneric hybrids of *Chrysanthemum morifolium* and *Ajaniaa przewalskii* and determined that the proline amounts increased in response to drought stress. Our findings on the proline contents demonstrate that the proline amounts increase in the pepino plant leaves in response to drought stress (Fig. 7). This result in agreement with the abovementioned findings. Plants may accumulate the proline for protection during drought stress in pepino and may be reduce the stress damage.

Drought stress caused lipid peroxidation and membrane impairment in plants (Nair *et al.,* 2008). MDA is end product of lipid peroxidation and it is considered a beneficial index for determination general lipid peroxidation (Hodges *et al.,* 1998). Tatar & Gevrek (2008) investigated the changes in lipid peroxidation, the RWC and the proline accumulation in wheat under water stress and noted that, in response to water stress, the MDA amounts in leaves of wheat increase. Yin *et al.* (2005) determined that the MDA contents in *Populus kangdingensis* increase in response to drought stress. Our findings show that the MDA contents in the pepino plant were higher in the stress group compared to the control (on the 36th day) (Fig. 8) indicates that the pepino can be affected by environmental stress factors and that the free radical formation in this plant can increase. The increase in the MDA contents in the membranes of the pepino plant in response to drought stress may be an indicator of oxidative damage as indicated another study (Ashraf *et al.,* 2010).

As a result, throughout the trial time (36 days) the RWCs and pigments (Chl a, Chl b, carotenoids) in the leaves of the pepino plant were found to have decreased in the stress groups compared to the control, whereas the total phenolic compounds, and the proline and the MDA contents were increased. Although the pepino plant increased the synthesis of some of its protective compounds (phenolics and prolines) in response to the drought stress, the pepino plant may be negatively affected by this type of stress. In further studies, we can analyze the antioxidant enzymes in pepino plants exposed to drought stress and increase the resistance in pepino plants by pretreatment of some substances.

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