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Role of Polyamines in Caprifig Drought Tolerance

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ABSTRACT

In this study, the role of polyamines in protection of *Ficus carica* L. against drought stress was investigated. Responses of four caprifig genotypes ('Dane Sephid', 'Khormaei', 'Pouz Donbali', and 'Shah Anjir') to alternate drought and irrigation periods were investigated. Drought stress significantly reduced membrane stability index (MSI) and chlorophylls content in the leaves of all caprifig genotypes. However, 'Shah Anjir' and 'Khormaei' genotypes were able to maintain both their MSI and chlorophyll contents at higher levels during drought stress. Drought stress significantly reduced caprifigs photosynthetic activity (PN), especially in the leaves of 'Dane Sephid' and 'Pouz Donbali' genotypes. The protective effect of carotenoids against photo damage to photosynthetic apparatus was proposed. However, it was concluded that anthocyanins may not be involved in caprifigs defensive mechanisms against drought stress. Higher ascorbic acid (AA) accumulation in the leaves of Shah Anjir and Khormaei under drought stress, suggested a possible action of AA as an antioxidant agent in drought tolerant caprifigs. Drought stress induced polyamines (PAs) accumulation in the leaves. Higher PAs in the leaves of 'Shah Anjir' and 'Khormaei' coincided with higher MSI and leaf relative water content in these two genotypes. It was concluded that higher PAs concentrations in drought tolerant genotypes are probably acting both as osmoprotectants and reactive oxygen species (ROS) scavengers. Based on the results, the caprifig genotypes used in this study were grouped in three categories: 1) drought tolerant ('Shah Anjir' and 'Khormaei'), 2) Semi-sensitive ('Pouz Donbali'), and 3) sensitive ('Dane Sephid').

Keywords: *Anthocyanin, Carotenoids, Leaf Water Potential, Leaf Ascorbic Acid Content, Leaf Relative Water Content, Photosynthetic Activity.*

1. INTRODUCTION

Water limitation is one of the most important factor in reducing agricultural crops production. Crops cultivated under Mediterranean climates usually are subjected to drought stress periods during growth season. Fig (*Ficus carrica* L.) is a major horticultural crop cultivated under Mediterranean climates. Extended periods of drought conditions adversely affect fig production in Asia (Gholami *et al.*, 2012). Improving drought tolerance traits in fig is of great importance in this economically important crop in arid and semiarid regions of the world. A better knowledge of plants responses to water deficit is also useful in drought resistance breeding programs.

Plants respond to water stress by altering cellular metabolism and invoking defense mechanisms (Bohnert and Jensen, 1996). Morphological changes and changes in physiological and biochemical processes help plants resist drought stress (Bohnert and Jensen, 1996; Chaves *et al.*, 2003).Polyamines (PAs) are multifunctional compounds which have been shown to be involvedin a wide range of biological processes in plant growth and development Putrescine (Put), spermidine (Spd) and spermine (Spm) are the most abundant PAs in plants. Their biological activity is attributed to their cationic nature (Galston *et al.*, 1997; Tiburcio *et al.*, 1993; Bias and Ravishankar, 2002). Changes in PA levels occur in response to a variety of abiotic stresses (Bouchereau *et al.*, 1999; Groppa and Benavides, 2008; Kusano, *et al.*, 2008; Alcázar*et al.*, 2010; Gill and Tuteja, 2010a). Remarkably, drought stress induces changes in PAs, which broadly correlate with drought resistance traits (Bouchereau *et al.*, 1999; Kusano, *et al.*, 2008; Gill and Tuteja, 2010b). Studies have suggested that PAs as membrane stabilizers, as osmolytes and as antioxidant agents may be involved in plants defense mechanism against environmental stresses (Bias and Ravishankar, 2002; Kaur-Sawhney*et al.*, 2003).

In order to increase our understanding of the role(s) that polyamines play in plants drought tolerance, in the present study we have analyzed the PAs profiles of several *F. carrica* L genotypes in response to a period of fluctuation in soil water availability.

2. MATERIAL AND METHODS

This study was conducted in the experimental greenhouse of the Department of Horticultural Science of Shiraz University, Iran during February 2011 to September, 2012. Plant materials used in this study were cuttings from four Iranian caprifigs namely 'Daneh Sephid', 'Pouz Donbali', 'Shah Anjir', and 'Khormaei'. Cuttings from these genotypes were collected in February 2011 and were allowed to root in sand medium. At the end of winter 2012, the rooted cuttings were transplanted into pots containing 12 kg of sand, leaf mold and loamy soil (1:1:1, v/v/v). Three months later, drought stress was applied to young plants during their growing period.

Drought stress was applied by withholding irrigation for 15 days. During this period, control plants were watered daily to keep their soils moisture content at the field capacity (FC). After 15 days of drought application, the stressed plants were irrigated to FC level and the rates of genotypes recovery were evaluated after 10 days.. The experiment was repeated twice and were analyzed at three stages: 1) at the beginning of the experiment, 2) at the end of the drought stress and 3) at the end of the drought recovery.

2.1. Leaf water content and membrane stability index

To evaluate the effects of the treatments on plants water content, by using ten 7 mm leaf disks, the leaves relative water contents (RWC) were determined. After taking the fresh weight (FW) of the leaf disks, they were then hydrated to saturation point for 48 h at 5ºC in darkness and weight again (TW). Leaf discs were dried in an oven at 105ºC for 24 h (DW). The leaves relative water content was calculated by the following expression (Filella*et al.*, 1998):

$$RWC\% = (FW - DW)/(TW - DW) \times 100$$

Membrane stability index (MSI) was used to assess membrane permeability. MSI was determined by an electrical conductivity meter using the method described by Lutts*et al.* (1995).

2.2. Net photosynthesis measurement

Leaf gas exchange was evaluated at the middle of the plants height. The measurements were taken between 12:00 and 13:30 PM. Photosynthesis parameters were measured using an open gas exchange system with a 4 cm^2 leaf cuvette (LC*i*, ADC, UK).

2.3. Measurement of chloroplasts pigments

Fully expanded young leaves taken from the middle of stem selected for the determination of chloroplast pigments. Leaves were ground with mortar and pestle, and the powdered tissues were extracted with an 80%acetone solution. The samples were centrifuged for 10 min at 5000 rpm. The chlorophyll and carotenoids contents were determined by spectrophotometer at 470, 646 and 663 nm according to the method described by Lichtenthaler (1987).

2.4. Anthocyanin measurement

Five hundred mg leaf material was homogenized in 1 ml of acidified (1% HCl) methanol and maintained at 4ºC for 24 h. The anthocyanin absorbance at 550 nm was measured by spectrophotometer. The concentration of anthocyanin was determined by using the extinction coefficient: ε_{550}= 33,000 (cm^2/mol.) (Wagner, 1979).

2.5. Leaf ascorbic acid content

Leaves ascorbic acid content was assayed as described by Omaye *et al.* (1979). One gram of fresh material was ground in a pestle and mortar with 5 mL of 10% tri chloro acetic acid (TCA), the homogenate was centrifuged at 3500 rpm for 20 minutes. The pellet was re-extracted twice with 10% TCA and the supernatant was increased to 10 mL and used for analysis. To 0.5 mL of the extract, 1 mL of DTC reagent (2,4-Dinitrophenyl hydrazine-Thiourea-$CuSO_4$ reagent) was added and mixed thoroughly. The tubes were incubated at 37°C for 3 hours and to this, 0.75 mL of ice cold 65% H_2SO_4 was added. The tubes were then allowed to stand at 30°C for 30 min and the absorbance of the solution was read at 520 nm by a spectrophotometer. The ascorbic acid content was determined using a standard curve and the results were expressed in milligrams ascorbic acid per gram leaf fresh weight.

2.6. Polyamines determination

Leaf tissues were homogenized in chilled mortar in a solution of 0.2 N $HClO_4$. The homogenates were centrifuged at 4°C in a clinical centrifuge. The supernatants were analyzed for polyamines by high-performance liquid chromatography (HPLC) as described by Smith and Davis (1985).HPLC analysis was carried out on an Agilent 1200 series (Agilent Technologies, USA), equipped with a Zorbax Eclipse XDB-C18 column (4.6 × 5 µm i.d.; × 150 mm film thickness, RP), and a photodiode array detector (PDA). Elution was monitored at 280 and 230 nm. The column temperature was 30 ºC. The injection volume was 20µL and it was done automatically using auto sampler.

2.7. Statistical analyses

The experiments were conducted as a complete randomized design with ten replications and statistical differences between measurements were analyzed following the analysis of variance ANOVA using SPSS 16.0 software. Duncan's multiple range test was used to compare means and differences ($P< 0.05$).

3. RESULTS

3.1. Effects on RWC

The leaves relative water content (RWC) was reduced significantly at the end of drought stress period (Fig., 1). The lowest was in Dane Sephid genotype. When irrigated, the RWC of stressed plants increased significantly. However, in Dane Sephid and Pouz Donbali genotypes, the RWC recovery was not as complete as that of control plants. Cell membrane stability index (MSI) showed significant decrease in the leaves of caprifig genotypes exposed to drought stress (Fig., 1). The lowest MSI values were found in the leaves of 'Dane Sephid' and 'Pouz Donbali'. Following the irrigation of drought stressed plants, their MSI's increased significantly. The increase for Shah Anjir and Khormaei genotypes was complete similar to control plants.

3.2. Effects on photosynthesis

Drought stress reduced the rates of photosynthesis in the leaves of caprifig genotypes significantly (Fig. 2). The lowest photosynthesis rate was found in the leaves of drought stressed 'Dane Sephid' and the highest in the leaves of 'Khormaei' genotype respectively. After re-

watering, the rate of photosynthesis was fully recovered to that of control level only in Khormaei genotype (Fig. 2).

3.3. Changes in chlorophyll and carotenoid contents

Drought stress affected leaves chlorophyll concentration of 'Dane Sephid', 'Shah Anjir' and 'Khormaei' significantly (Fig. 3). Chlorophyll concentration increased in the leaves of 'Shah Anjir' and 'Khormaei' but decreased in the leaves of 'Dane Sephid' significantly. After irrigation, the leaves chlorophyll content reached to the control level.

Changes of leaves carotenoids contents are shown in fig. 4. Carotenoids were significantly reduced in the leaves of 'Dane Sephid' and 'Pouz Donbali' by drought stress; however, it was significantly increased in the leaves of 'Shah Anjir'. Drought stress did not affect carotenoids content in the leaves of 'Pouz Donbali'. Chlorophylls to carotenoids ratio was significantly reduced in the leaves of 'Dane Sephid' after the drought period, however it was significantly higher in the leaves of 'Khormaei' (Fig. 4). With the exception of 'Dane Sephid', the chlorophylls to carotenoids ratios were recovered to the control levels in the leaves of fig genotypes after the re-watering phase.

3.4. Leaves anthocyanin contents

Leaves anthocyanin concentration was significantly reduced by drought stress (Fig. 5). Except in Shah Anjir genotype, anthocyanin concentration in the leaves of other genotypes did not fully recover after the re-watering phase.

3.5. Leaves ascorbic acid (AA)

Leaves ascorbic acid (AA) concentration was significantly increased after the drought period (Fig. 6), and the highest AA level was found in the leaves of 'Khormaei'. Leaves AA contents of the stressed plants was significantly reduced after the re-watering phase; however, the AA content of 'Dane Sephid' remained unchanged after the re-watering phase.

3.6. Leaves polyamines contents

Putrescine (Put) concentration was increased in the leaves of drought stressed caprifig genotypes (Fig. 7) and the highest levels were found in 'Khormaei' and 'Shah Anjir' respectively. After the re-watering period, Put concentration was reduced in the leaves of stressed plants. Spd concentration remained unchanged during the experiment (Fig. 7); however, reducing trends in Spd contents were found in the leaves of 'Pouz Donbali', 'Shah Anjir', and 'Khormaei' under drought stress. The amount of Spm levels in the leaves of all caprifig genotypes increased under drought stress significantly (Fig. 7). Spm levels were significantly higher in the leaves of 'Dane Sephid' and significantly reduced in the leaves of drought stressed fig genotypes after the re-watering period. Fig. 7 shows changes of total PAs concentration in the leaves of caprifig genotypes during the experiment. PAs concentration significantly increased after drought stress and the highest level was found in the leaves of 'Pouz Donbali', 'Shah Anjir', and 'Khormaei' genotypes respectively. PAs concentrations were recovered to the control levels after the re-watering phase.

4. DISCUSSION

In the present study, the RWC offig leaves significantly reduced in response to drought stress which will result in significant loss of leaves cell turgor pressure. The wilting of leaves and shoot apexes are usually the first signs of leaves cells turgor loss which appeared first in 'Dane Sephid' first. The wilting leaves and shoots apex are the usual signs of turgor loss which were observed first in 'Dane Sephid' genotype. Stomatal closure as a result of water loss will induce the formation of reactive oxygen species which will damage cell membranes and photosynthetic apparatus (Bian and Jiang, 2009). Rostami and Rahemi (2013) showed that drought stress induced oxidative stress causing massive injuries to leaf tissues of caprifig genotypes. MSI data are widely used to assess cell membrane stability and integrity under environmental stresses (Karimi and Rahemi, 2012). In the present study, MSI was reduced at the end of the drought stress phase. Reduced MSI indicates the loss of selective permeability of cell membranes (Grzesiak *et al.* 2007) and the differences in MSI values among the caprifig genotypes can be used as a criterion to differentiate between sensitive and tolerant genotypes as far as the maintenance of membrane integrity is concerned. Rapid repair of cell membrane damages is critical for plants to tolerate periodic drought stresses. Lack of such membrane recoveries were found in the leaves of 'Dane Sephid' and 'Pouz Donbali' after rehydration the period (Fig.1). Bukhov *et al.* (1990) stated that drought resistant plant species avoid loss of chloroplasts membrane integrity. Hence, higher rates of net photosynthesis (P_N) may be expected in the leaves of drought tolerant genotypes exposed to water deficit.

The results of the current study showed that drought stress can significantly reduce P_N especially in the drought sensitive caprifig genotypes (Fig.2). The reduction in P_N as a result of water stress can be attributed to both stomatal and non-stomatal factors (Shangguan *et al.*, 1999). Genotypic differences in photosynthesis of caprifigs were suggested based on the results of the current study. $P_N$data showed that the inhibition in photosynthesis and other related traits was more pronounced in susceptible genotypes than in the relatively tolerant ones. Maintaining P_N at higher levels under severe water stress can be attributed to higher chloroplasts capacity to fix CO_2 and less structural damages under water deficit condition (Herppich and Peckmann 1997). Low MSI at the end of the drought period represents the extent of damages to cell membrane and to organelles structures such as chloroplasts in the drought sensitive genotypes.

Damages to chloroplasts structures by ROS and/or photo degradation of the pigments probably have led to loss of chlorophylls under drought stress (Anjum *et al.*, 2011). Chlorophyll concentration in the leaves of 'Dane Sephid', the most sensitive genotype, was reduced under drought stress. However, it was increased in the leaves of 'Shah Anjir' and 'Khormaei'. Maintaining the integrity of chlorophyll molecules under drought stress has also been reported by Kraus *et al.* (1995) and Sairam *et al.* (1998) as a criterion for drought stress tolerance. Enzymatic and non-enzymatic antioxidants which act as ROS scavengers have been found in the chloroplasts. Carotenoids, anthocyanins, and ascorbic acid are the non-enzymatic compounds which protects chlorophylls against oxidative stress under drought stress (Muller *et al.*, 2006). According to Rostami and Rahemi (2013), increased

chlorophyll concentrations in the leaves of 'Shah Anjir' and 'Khormaei' may be attributed to higher activity of antioxidant enzymes in the leaves of drought tolerant caprifig genotypes.

Ascorbic acid (AA) protects cell membrane and internal organells against oxidative stress by scavenging super oxide radicals and hydrogen peroxide directly (Gill and Tuteja, 2010). In the current study drought stress induced AA accumulation in the leaves of fig genotypes (Fig.6). Increase in AA content in the leaves of drought stressed plants has been reported previously (Sairam and Sirvastava, 2001; Shi-sheng and Min-wen, 2007). We found higher accumulation of AA in the leaves of 'Shah Anjir' and 'Khormaei', the two drought tolerant genotypes. Sairam and Sirvastava (2001) and Nair *et al.* (2008) also found higher accumulation of AA in the leaves of drought tolerant plants.

The carotenoids contents of caprifig leaves genotypes respond differently to drought stress. However, higher carotenoids contents were found in the leaves of the drought tolerant genotypes. Carotenoids compounds as quenchers of singlet oxygen molecules, protect chloroplasts against photodynamic damage (Nishida *et al.,* 2007). Thus, higher carotenoids in the leaves will result in less structural damage and higher chlorophyll concentration in the drought tolerant figs. Chlorophylls to carotenoids ratios suggest that the amount of carotenoids should be higher than 3.0 ug/g FW to be effective in protecting chlorophyll molecules against photodynamic damages. Our results are in agreement with those reported by Sircelj *et al.* (2007) for apples. The higher ratios of chlorophyll to carotenoids indicate the role of carotenoidsin protecting the photosynthetic apparatus (Loggini *et al.,* 1999).

Besides their roles in protecting chloroplast against photodynamic damage, anthocyanins have also been reported to act as potent antioxidant (Edreva *et al.,* 2008). Neill (2002) showed that anthocyanins could provide widespread cellular protection for cellular membranes, organelles, and DNA. In our study, leaf anthocyanin concentration reduced under drought stress. This is in agreement with the findings reported by Jung (2004). After the drought phase, higher concentration of anthocyanins found in the leaves of the drought sensitive genotypes, 'Dane Sephid' and 'Pouz Donbali'. It can be stated that anthocyanins may not have important role in photo-protection of figs as the carotenoids.

Effect of drought stress on concentration of the polyamines was somehow inconsistent. Although the amount of Spd was not affected by drought stress, the concentration of total PAs increased in response to drought stress (Fig.7) which was mainly due to Put and Spm accumulation in the leaves of drought stressed fig genotypes. These results are in agreement with those reported by Kusano, *et al.* (2008) and Gill and Tuteja (2010). In the present study, the lowest concentration of PAs was found in the leaves of 'Dane Sephid', which is the most sensitive fig genotype to drought stress. Studies made by Lee (1997) revealed that stress tolerant plants endogenously have more PAs than drought sensitive ones. Furthermore, transgenic plants overproducing PAs are more drought tolerant (Galston *et al.,* 1997).Exogenous application of PAs confers protection against abiotic stresses (Basra *et al.,* 1997; Nayyar and Chander, 2004, Karimi and Rahemi, 2012).The data on concentrations of PAs in the leaves of control plants revealed that differences in PAs content in the leaves of caprifigs is primarily caused by genotypic differences. The differences in PAs levels in the leaves of drought stressed caprifigs is probably correlated with the plants ability to preserve leaf water content during water stress. However, in 'Dane Sephid' genotype, low concentrations of PAs, might have been due to heavy loss of water affecting the enzymes involved in PAs synthesis pathway. Chen and Kao (1993) and Erdei *et al.* (1996) stated that PAs accumulation under drought stress may contribute to osmoregulation and improved water preserving status under drought stress. However, our data revealed that the amount of PAs in the fig genotypes was too low to act as osmoprotectants.

PAs exert a positive role in the photosynthesis of plants in response to various environmental stresses. Chloroplasts and Thylakoids membranes especially those associated with PSII are enriched in polyamines (Kotzabasis *et al.,* 1993; Navakoudis *et al.,* 2003). This shows a potential role of polyamines in maintaining plants photosynthetic efficiency. In the present study, higher rates of P_N are probably due to the higher amounts of PAs which protect chloroplasts against the damaging effects of antioxidants produced under drought stress.

4. CONCLUSION

It is concluded that drought tolerance in caprifigs genotypes may be associated with the maintenance of normal cell membrane functioning and integrity under severe water stress. Our data suggest that higher activity of antioxidants in the leaves of caprifig genotypes may help the plants to preserve chlorophylls under drought stress. Significant increase in the chlorophylls concentration which was observed in the leaves of drought stressed caprifigs, may be correlated with leaves dehydration coinciding with the increase in antioxidants activity and other photodynamic damage protecting compounds. We found that carotenoids are more important in photo protection in caprifig leaves. However, the results suggest that to be effective as photo damage protecting agents, the amount of carotenoids should be higher than a threshold value in caprifig leaves. Based on our findings, higher concentrations of AA and PAs are critical in protecting caprifig genotypes against oxidative stress induced under water deficit conditions.

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FIGURES

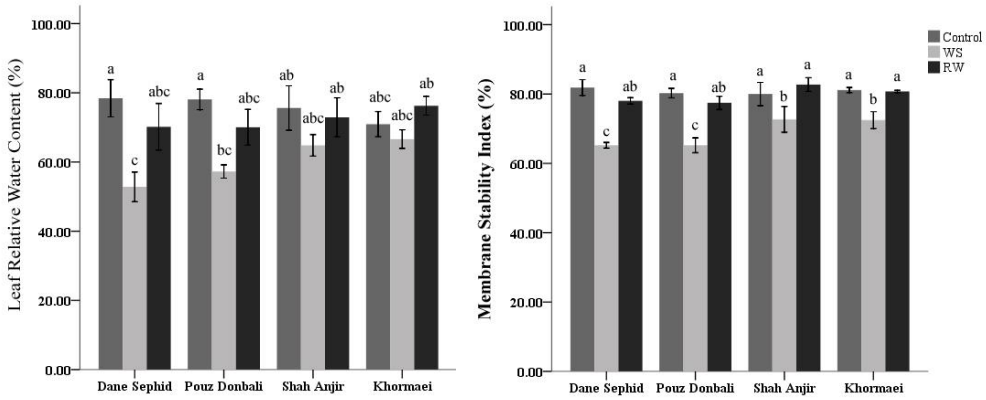

Figure 1. Leaf relative water content (RWC) and Cell membrane stability indices (MSI) changes during water stress (WS) and re-watering (RW) periods.

† Means denoted by the same letter did not significantly differ at *P* <0.05 according to Duncan's multiple range test.

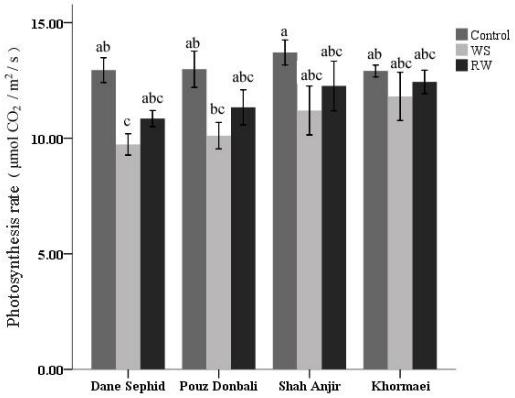

Figure 2 Changes in rates of photosynthesis during water stress (WS) and re-watering (RW) periods.
† Means denoted by the same letter did not significantly differ at *P* <0.05 according to Duncan's multiple range test.

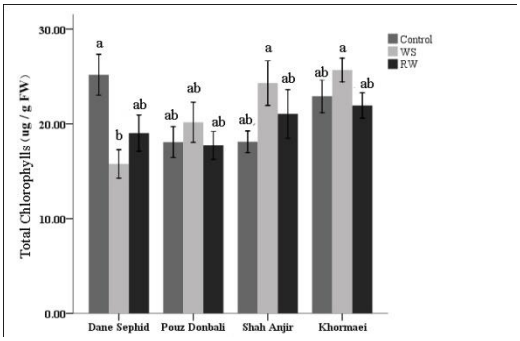

Figure 3 Changes in leaves chlorophyll content during water stress (WS) and re-watering (RW) periods
† Means denoted by the same letter did not significantly differ at *P* <0.05 according to Duncan's multiple range test.

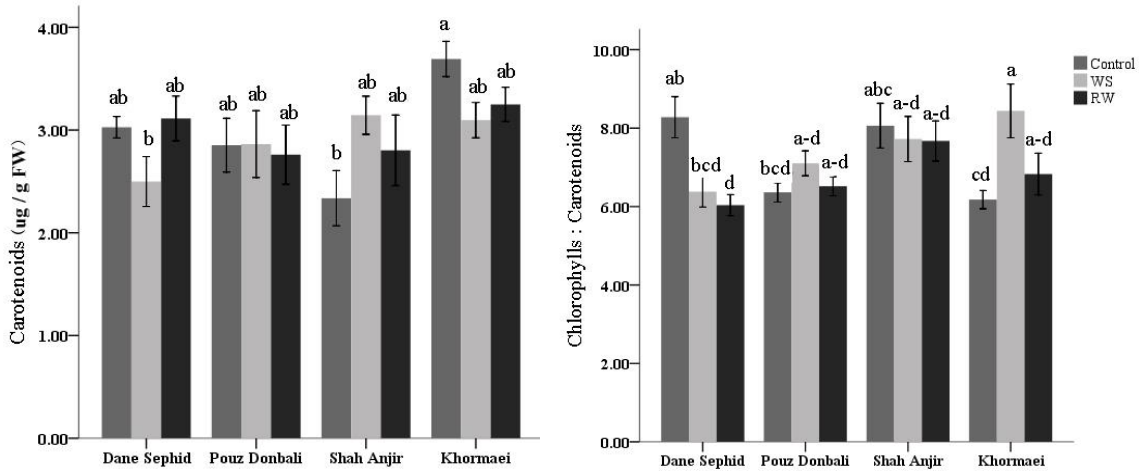

Figure 4 Leaf carotenoids concentration and changes in carotenoids: chlorophyll ratio during water stress (WS) and re-watering (RW) periods.
† Means denoted by the same letter did not significantly differ at *P* <0.05 according to Duncan's multiple range test.

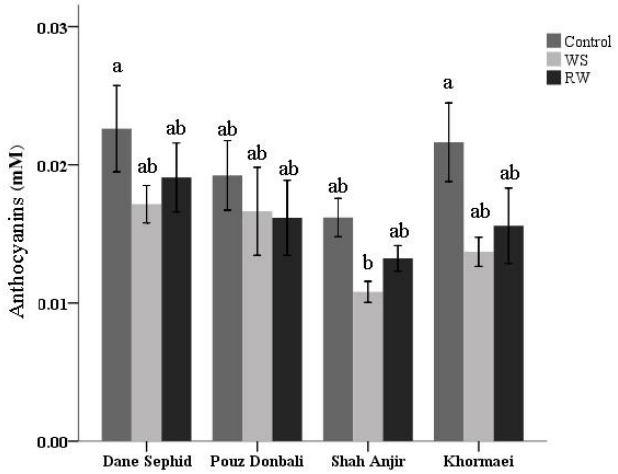

Figure 5 Changes in the leaves anthocyanins concentration during water stress (WS) and re-watering (RW) periods. †Means denoted by the same letter did not significantly differ at *P* <0.05 according to Duncan's multiple range test.

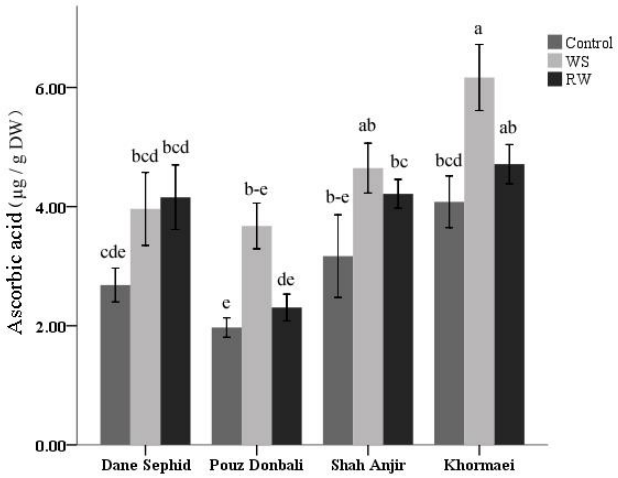

Figure 6 Changes in leaves ascorbic acid concentration during water stress (WS) and re-watering (RW) periods. †Means denoted by the same letter did not significantly differ at *P* <0.05 according to Duncan's multiple range test.