



## Investigation of proline, total protein, chlorophyll, ascorbate and dehydroascorbate changes under drought stress in Akria and Mobil tomato cultivars

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### Abstract

In this study the effects of drought stress on proline, protein, chlorophyll a/b, ascorbate and dehydroascorbate were investigated in Akria and Mobil cultivars of tomato species. The seeds were cultured at 23 °C with 15-16 hours light period and irrigation was done based on field capacity control (FC), mild drought stress ( $\frac{2}{3}$  FC) and severe drought stress ( $\frac{1}{3}$  FC) under greenhouse conditions. After nine weeks of sowing, leaf, stem and root were isolated and studied for various indices. The results indicated that amount of proline increased significantly in mild and severe stresses in root, but in stem and leaf proline increased only under severe drought stress. Chlorophyll a and b and total protein decreased significantly in mild and severe stress in leaf, stem and root. Ascorbic acid (ASC) increased but dehydro ascorbate decreased significantly in both species. It could be concluded that in both cultivars proline and ASC content increased under severe and mild drought stress although soluble protein and chlorophyll a/b decreased under severe stress. Therefore, in tomato plants osmolyte such as proline and also ASC as an antioxidant compound increased against drought stress. Protein accumulation has protecting role under mild stress.

**Keywords:** proline; chlorophyll a/b; total protein; ascorbate; dehydroascorbate; Akria cultivar; Mobil cultivar

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### Introduction

Among various abiotic stresses, drought is one of the basic factors for restricting crops production (Vallivodan and Nguyen, 2006). In fact, it is predicted that one third of world population will be threatened by water shortage in year 2025.

Proline is one of the protective molecules that can unite oxygen and free radicals caused by stress. Therefore, one of the roles of proline in tomato shrubs is probably reacting against drought stress (Behnamnia et al., 2009). Proline's role as an osmotic factor is already established (Kavir kishor et al., 2005) and low water stress increases proline contents in plants. A relatively recent study on tomato found that use of brasino estroid in two stress levels (mild and severe) increased the amount of proline 3 and 4 time in comparison with control. The effect of drought

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stress was also investigated on ABA (abscisic acid) and proline in different *Zea mays* species and close correlation was found between proline accumulation and ABA with drought stress (Heidari and Moaveni, 2009).

Although plant resistant mechanisms are not known clearly, new proteins accumulation and stress genes expression that code biosynthetic enzymes against osmotic stress were investigated (Vallivodan and Nguyen, 2006). Also a study on gene proteins have shown that osmotic proteins increased in low water stress (Hajheidari et al., 2005). The quantity of aquaporin existing in plasma membrane regulated membrane hydrolytic function and increased water permeability under environmental factors like low water, hormones and light conditions (Yamada et al., 1995). Water shortage trigger to LEA proteins production that their work hasn't identified completely however, some evidence indicated that these proteins play role in increasing plant resistance against drought stress (Wise and Tunnacliffe 2004).

Ascorbate and dehydroascorbate are important plant antioxidants. They act as a reactive oxygen and eliminator of hydroxyl radicals, preventing free radicals chain reactions under drought stress conditions. Actually, the existence of these substances causes biological defensive mechanisms in plants in dry regions and under low water regimes. Ascorbate content was reported to increase in plants during drought stress and this compound is believed to play a vital role in detoxifying reactive oxygen species and free radicals (Inze and Montago, 2002).

Reduction of chlorophyll a/b ratio in resistant tomato under low water condition indicated that drought stress changes the amount of chlorophyll in plant (Tewari et al., 2002; Caretto et al., 2002). Reduction of chlorophyll was due to chloroplast decomposition and disappearing thylakoid structures (Cornoy et al., 1988).

The aim of this study was investigating the effects of mild and severe drought stress on proline, total protein, chlorophyll a/b, ascorbate and dehydroascorbate content in two tomato cultivars, namely, akria and mobil under greenhouse condition.

## Materials and Methods

The project was done during March 2008 in experimental field in Ashtian town (48° 57' longitude 33° 30' latitude and 2109 hight). The seeds of two tomato cultivars, akria and mobil, were obtained from Seed and Plant Improvement Institute, Karaj, Iran. Seeds were sown in pots with 15 cm diameter and 25 cm length containing a mixture of fertilized peat, clay and sand (1/1/2 v/v). All pots were kept in greenhouse conditions with 23±1°C during darkness and 26±1 °C in 15-16 hour light period with photon flux density ranging between 500 and 600  $\mu\text{Mm}^{-2}\text{s}^{-1}$  and 70% relative humidity. Irrigation was done daily during the first week and feed with 50 ml Long Ashton solution and in subsequent of experiment, seedling irrigated twice a week for one month. The treatment was applied after one week of culturing. The amount of irrigation was determined based on soil field capacity. The first group, i.e., control was irrigated according to field capacity (FC), the second group received mild stress ( $\frac{2}{3}$  FC) and the third group received severe stress ( $\frac{1}{3}$ FC). The control plants received 300 ml water every 2-3 days and the mild and severe drought stress treatments involved 200 ml and 100 ml water every 2-3 days, respectively. The plants were placed in 3 rows with 4 replications for each treatment.

### Proline assay

Proline was estimated by Bates et al. (1973) method. Samples were homogenized in 10 mL 3% (w/v) sulfosalicylic acid, and proline was assayed by the acid ninhydrin method. The absorbance was measured spectrophotometrically at 520 nm. Proline was calculated based on  $\mu\text{M} \cdot \text{g}^{-1} \cdot \text{FW}$ .

### Ascorbic acid and dehydroascorbate assay

The amount of ascorbic acid was estimated using de Pinto et al. (1999) method. 0.5 g of leaf was homogenized in metaphosphoric acid 5% (w/v) and centrifuged for 15 minutes. 0.3 ml of solution was mixed with 0.75 ml  $\text{KH}_2\text{PO}_4$  buffer and 0.3 ml distilled water and kept in room temperature for 10 minutes. Then, 0.6 ml TCA

10% (W/v), 0.6 ml orthophosphoric acid 44% (w/v), 0.6 ml dipyrindil ( $C_{10}H_8N_2$ ) 4% (w/v) and 10  $\mu$ l  $FeCl_3$  were added and shaken for 20 min in 40 °C.

The same method was used in order to measure dehydroascorbate content. 0.5 g of leaf was homogenized in metaphosphoric acid 5% (w/v) and centrifuged for 15 minutes. 0.3 ml of solution was mixed with 0.75 ml  $KH_2PO_4$  buffer and 0.15 ml DDT and kept in room temperature for 10 minutes. Then, 0.15 ml NEM 5% (w/v) was added and again kept in room temperature for 10 minutes. Then, 0.6 ml TCA 10% (w/v), 0.6 ml orthophosphoric acid 44% (W/V), 0.6 ml dipyrindil ( $C_{10}H_8N_2$ ) 4% (w/v) and 10  $\mu$ l  $FeCl_3$  were added and shaken for 20 min in 40 °C. The absorbance was measured spectrophotometrically at 525 nm wavelength for both of them. Ascorbate and dehydroascorbate concentrations were calculated based on  $mgg^{-1}$  FW.

### Leaf chlorophyll assay

Total chlorophyll content was determined using Lichtenthaler and Wellburn method (1983). 0.05 g of fresh leaf was extracted in 10 ml 80% acetone (v/v). The absorbance of the extracts at 663 and 645 for chlorophyll a and b were then measured using a UV/visible spectrophotometer (UV-2450, Shimadzu Corporation, Tokyo, Japan), Chlorophyll content was estimated based on  $mgg^{-1}$  FW.

### Total protein assay

Protein was estimated by Lowry et al. (1951) method. Reagent A: 1%  $Na_2CO_3$  in 0.5 N NaOH; Reagent B: 1%  $CuSO_4 \cdot 5H_2O$ ; Reagent C: 2% sodium tartrate ( $Na_2C_4H_4O_6$ ); Reagent D: Mix 0.5 ml reagent C with 0.5 ml reagent B and 10 ml reagent A and Reagent E: Folin 0.2 N.

Soluble proteins were extracted from 2 g dry weight of each sample into 5 ml Tris-HCl buffer (pH=8.0) containing 26.8 ml 0.2 N HCl 17.2% sucrose, 1% ascorbic acid and was then centrifuged. 1 ml of reagent D was added into 0.05 ml of resulted solution and kept in temperature room. Then, 3 ml of reagent E was added and the sample was kept in Bain-marie at 50 °C. The absorbance was measured

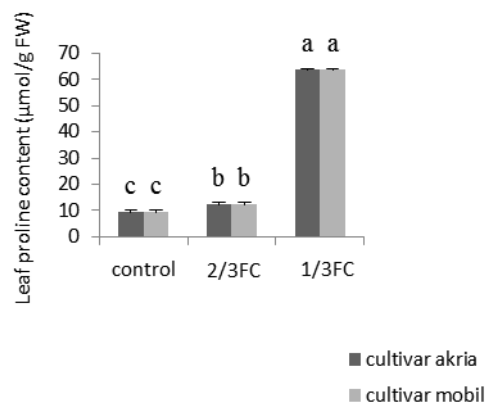


Fig. I. The effect of different drought stress treatments on proline content in tomato leaf based on Duncan test  $P \leq 0.05$ ; the averages are related to 4 replications.

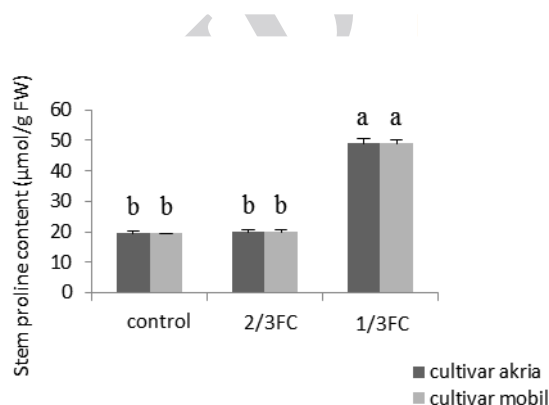


Fig. II. The effect of different drought stress treatments on proline content in tomato stem based on Duncan test  $P \leq 0.05$ ; the averages are related to 4 replications.

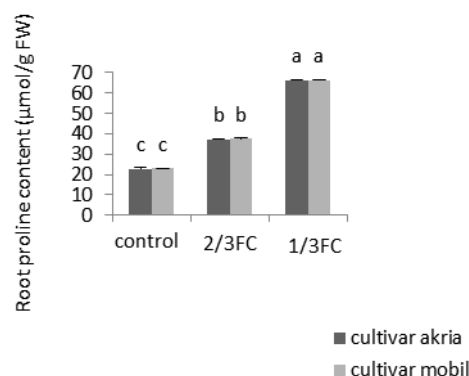


Fig. III. The effect of different drought stress treatments on proline content in tomato root based on Duncan test  $P \leq 0.05$ ; the averages are related to 4 replications.

spectrophotometrically at 625 nm. Protein was calculated based on  $\mu$ M  $g^{-1}$  FW.

## Statistical analysis

The experiment was done based on completely randomized block design with four replications for each treatment. One-way analysis of variance (ANOVA) was performed and means comparison was analyzed using multiple range Duncan test. Collected data were analyzed using SPSS software (version 15). The charts were drawn using Excel software.

## Results

### Proline

Leaf proline increased significantly under mild and severe drought stress in comparison with control in both species (Fig. I). The amount of stem proline was affected by two mild and severe stresses. However, there was no significant difference with control in both species. On the other hand, there was a significant increase in the stem proline content under severe stress (Fig. II). Proline content was significantly increased under both mild and severe drought stress in comparison with control plants (Fig. III).

### Leaf total protein

As Fig. (IV) indicates, leaf total protein content was significantly different in both severe and mild stress treatments compared with control. This protein reduction is affected by mild and severe drought stress in both cultivars and both cultures. Also significant differences existed between control and both severe and mild stress treatments in relation to total protein (Fig. V). This effect was similar in both cultivars. Moreover, while there was no significant difference between control and mild stress groups (Fig. VI), the difference between both mild and severe drought stress treatments and the control group in both cultivars was significant and the amount of root protein reduced in roots under severe drought stress.

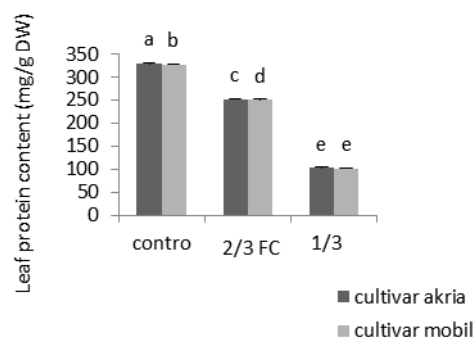


Fig. IV. The effect of different drought stress treatments on total protein in tomato leaf based on Duncan test

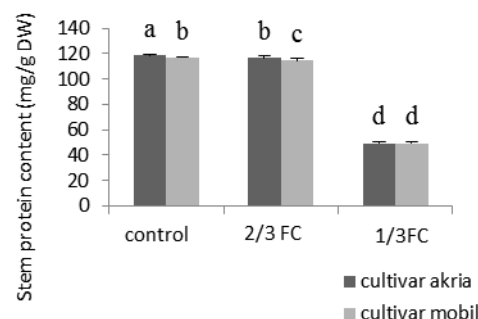


Fig. V. The effect of different drought stress treatments on total protein in tomato stem based on Duncan test  $P \leq 0.05$ ; the averages are related to 4 replications.

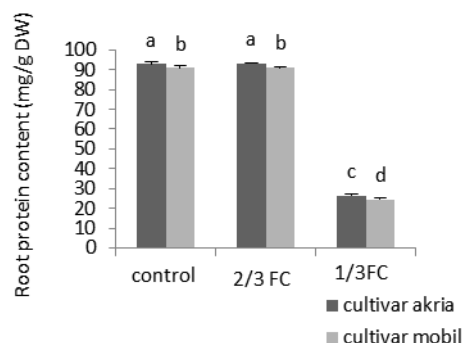


Fig. VI. The effect of different drought stress treatments on total protein in tomato root based on Duncan test  $P \leq 0.05$ ; the averages are related to 4 replications.

## Chlorophyll a and b and total chlorophyll content

The amount of Chlorophyll a decreased significantly in both levels of drought treatment. There was no difference between cultivars. Also, Chlorophyll b content was decreased significantly in both mild and severe treatments (Figs. VII and VIII). Total Chlorophyll content was reduced in mild and severe stresses and reduction in mild stress was the same as in severe stress (Fig. IX)

## Ascorbate and dehydroascorbate content

Leaf ascorbate was increased in mild and severe drought stresses and the amount of ascorbate was higher in severe drought stress (Fig. X). Although there was no difference between control and mild stress in relation to dehydroascorbate content, the amount of dehydroascorbate decreased in severe stress (Fig. XI).

## Discussion

Biochemical and physiological changes occur in response to low water condition in different plants. The increase of free proline occurs in decrease in water supply (Zhang et al., 2006). The synthesis of proline in plants extensively protects cell membrane and protein content in plant leaves. The synthesis and storage of osmolites differs in various plants. The amount of proline in rice (*Oryza sativa* L.) was increased steadily in salt stress using 24-epibassinolide which causes proline gene expression (Ozdemir et al., 2004). The results of this study are in agreement with other investigations (Behnamnia et al., 2009; Zhang et al., 2006). Proline acts as an osmolite beside enzymes and other macromolecules, and therefore, protects the plant against low water potential and causes osmotic regulation in plant organs. Also proline can act as an electron receptor preventing photosystems injuries in dealing with ROS function. Proline accumulation facilitates the permanent synthesis of soluble substances in closing stomata. This process is different in various plants. Proline accumulation in foliage

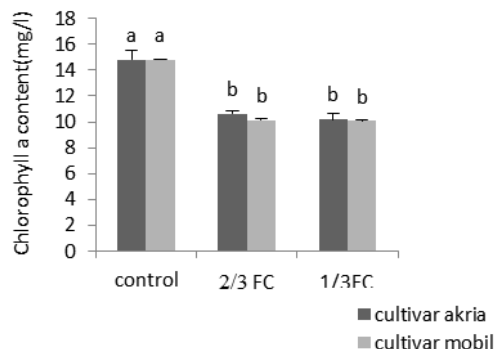


Fig. VII. The effect of different drought stress treatments on chlorophyll a in tomato leaf based on Duncan test  $P \leq 0.05$ ; the averages are related to 4 replications.

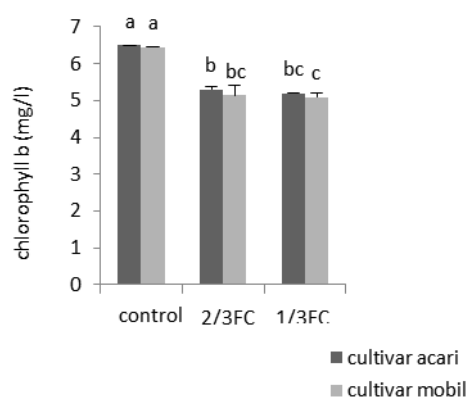


Fig. VIII. The effect of different drought stress treatments on chlorophyll b in tomato leaf based on Duncan test  $P \leq 0.05$ ; the averages are related to 4 replications.

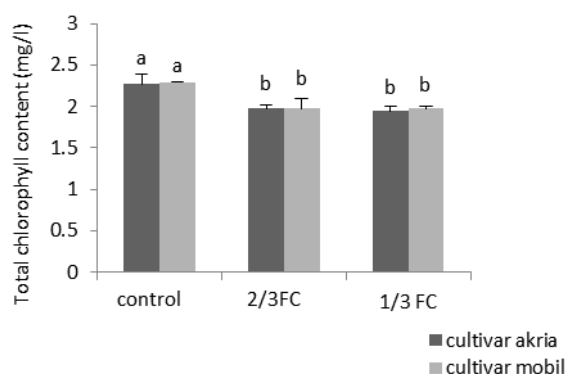


Fig. IX. The effect of different drought stress treatments on total chlorophyll in tomato leaf based on Duncan test  $P \leq 0.05$ ; the averages are related to 4 replications.

was seen in olive cultivars (*Olea europea* L.) in low water condition (Bacelar et al., 2009).

During drought treatment proteins were highly accumulated and after rehydration they returned to the control level (Chen and

Tabaeizadeh, 1992). The synthesis and accumulation of protein was investigated in two *Zea mays* species and remarkable changes were noticed in 78 out of 413 proteins of leaves. These evidences indicated that a kind of relationship existed between protein accumulation and plant physiological resistance against drought stress (Riccardi et al., 1998). Protein accumulation was seen in roots and leaves of two cultivars of *Zea mays*, so protein can protect plants from losing more water. Total soluble proteins were first increased and then decreased under drought stress in roots and leaves of two cultivars of *Zea mays* (Riccardi et al., 1998). In another study, the amount of soluble protein decreased in roots and leaves of two *Zea mays* cultivars (Havaux et al., 1987). This reduction depended on the intensity and duration of stress. The researchers speculated that the initial increase of proteins in drought stressed plants was related to stress proteins but the reduction occurred in next stage was due to the reduction in the amount of photosynthesis (Havaux et al., 1987). In another experiment, the leaf proteins increased during severe drought (Jiang and Huang, 2002). This did not correspond with the findings of the present study on tomato plants where total protein decreased significantly during mild and severe drought stresses.

Low water stress causes increase in ascorbate. Ascorbic acid is an important non enzymatic antioxidant that plays a role in regulating oxidation-reduction system in cells and protects them against stress. Ascorbic acid decreased oxygen radicals and reproduced  $\alpha$  tocopherol. Ascorbate peroxidase oxidizes Ascorbic acid and converts it to dehydroascorbate. So the ASA/DHAS ratio and oxidation-reduction cycle regulate gene expression and activate the oxidation-reduction enzymes factors (Yang et al., 2007). In another study on the effect of brassinosteroid on tomato shrubs, it was indicated that low water stress increased ascorbate extremely and the amount of dehydroascorbate also increased (Behnamnia et al., 2009). However, in the present study dehydroascorbate decreased significantly with severe drought stress and our findings did not support the previous investigation. In this study, the amount of ascorbate increased significantly

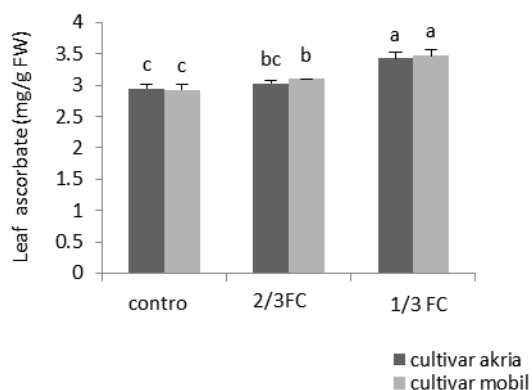


Fig. X. The effect of different drought stress treatments on ascorbate in tomato leaf based on Duncan test  $P < 0.05$ ; the averages are related to 4 replications.

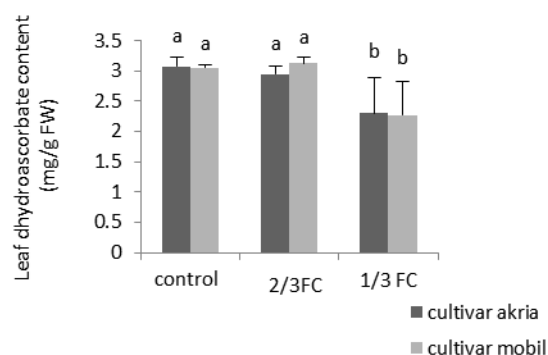


Fig. XI. The effect of different drought stress treatments on dehydroascorbate in tomato leaf based on Duncan test  $P \leq 0.05$ ; the averages are related to 4 replications.

under severe drought stress; in mild stress this increase was seen and this result corresponded with previous investigation.

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. Results of the present study showed that total chlorophyll a and b decreased under drought stress in both cultivars. The reduction in chlorophyll a/b under drought stress was also reported in some plants like sunflower and wheat (Synnerri et al., 1993; Pastori and Trippi, 1993).

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