

## Effect of Drought Interactions with Ascorbate on Some Biochemical Parameters and Antioxidant Enzymes Activities in *Dracocephalum moldavica* L.

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**Abstract:** In order to investigate ascorbic acid effect and interaction with on biochemical properties and antioxidant enzymes activities of *Dracocephalum moldavica* L. (Moldavian dragonhead) in drought stress ambient conditions, a factorial experiment in completely randomized with four replications, The main factor was irrigation in three level include without stress (FC), moderate stress (2/3FC) and severe stress (1/3 FC) and ascorbate operating at two levels (0 and 10 mmol) were considered. During, biochemical traits such as proline accumulation, whole carbohydrate, phenol compounds such as, anthocyanins and flavonoids, as well as catalase, peroxidase and ascorbate peroxidase activities were measured. Result showed that ascorbic acid treatment had positive and significant effect on measured traits of *Dracocephalum moldavica* L. Under stress condition plant *D. moldavica* with osmotic regulation mechanism and increasing of proline (112.43  $\mu\text{mol/L}$ ) and whole carbohydrate, to a degree tolerate stress condition. this result shows that ascorbic acid noticeably decreased deleterious effect of drought stress. soluble and insoluble carbohydrate and phenol compounds, anthocyanins and flavonoids, as well as catalase, peroxidase and ascorbate peroxidase activities significantly increased under severe stress. Using ascorbic acid under stress condition had positive effect on these traits and decreased stress effect and approach plants to control condition.

**Key words:** Antioxidant enzymes • Ascorbate • *Dracocephalum moldavica* L. • Drought stress • Proline • Sugars

### INTRODUCTION

*Dracocephalum moldavica* L. is an annual aromatic resistant plant with green leaves and branches belonging to the *Lamiaceae* family. The origin of the plant is reported to be south of Siberia and Himalayan hillsides. Its compounds are sedative and used as appetizer. Its extract has antibacterial effects and is used as a carminative, for stomachache and also in food and beverage industry, cosmetics and hygienic industry [1].

Most parts of Iran are located in regions where annual precipitation does not meet the necessary water for agricultural purposes. Thus, drought stress is unavoidable and to achieve favorable crop performance, water shortage should be compensated for by irrigation. Instability of ecological conditions such as water supply and genetic diversity of the plant species make them react

to the changes in different ways. Water supply is one of the most important factors that can influence plant's structure and its performance [2].

Under drought stress, a plant experiences physiological and biochemical changes. Some of these changes include accumulation of ABA, blocking and reduction the pores in the leaf area. Water stress induces accumulation of soluble compounds such as glycerol, sugars and proline. Accumulation of free proline might be an adaptation measure taken by the plants faced with drought stress. This compound increases osmotic pressure in plants [3]. Protective mechanism of proline has not been clearly explicated yet; however, this compound mainly plays a role in reducing water potential, stabilizing macromolecules and sweeping free forms of oxygen [4]. It is further reported that drought stress causes many changes in the carbohydrates content of the plants.

Also it is shown that as the drought stress increases, the starch content of the plants decreases. Under drought stress conditions proteins are destructed more rapidly [5].

In living cells, active forms of oxygen are produced during natural metabolism. Natural levels of antioxidants are normally sufficient for treating free radicals as they are turned into harmless metabolites. In fact, antioxidant enzymes and antioxidant compounds neutralize these free radicals. It should be noticed that free radicals are extremely harmful to the plant cells since they inactivate photosynthesis enzymes [6]. Compounds such as ascorbic acid [7] and salicylic acid [9] through boosting the plants' antioxidant activities are capable of mitigating damages due to drought stress. These damages on the cell membrane are caused by production of oxygen radicals [3].

There is a strong relationship between catalase, peroxidase and ascorbate peroxidase activities and phenol compounds content of the plants [10]. Some metabolites, e.g., carotenoids, flavonoids, anthocyanins and ascorbate protect the plant from oxidative stresses by sweeping and inactivating free radicals [10]. In a study difference was observed in glycoside compounds among several varieties of soybean plant [12]. These researchers found special glycoside compounds that reduce photosynthesis and plant growth. Phenol compounds particularly flavonoids and anthocyanins, because of their strong antioxidant nature, trap free antioxidants and reduce oxidative stress. They also reduce the damage by controlling oxidation macromolecules and damaged DNA [2]. In yet another study, ascorbate in soybean increased the content of phenol compounds while it decreased flavonoids and anthocyanins [4]. observed that phenol content in olive reduced with the increase of irrigation [16]. believed that this could be attributed to the hypothetical interference between both antioxidant groups of compounds (total phenol compounds and ascorbate). Ascorbate reduces flavonoids while total phenol compounds are increased under effect of ascorbate [16].

The present study aims at investigating the effects of drought stress on antioxidant compounds of *Dracocephalum moldavica* L. The study also focuses on the effect of an antioxidant such as ascorbate on plant's resistance to stress conditions.

## MATERIALS AND METHODS

The study was conducted in Pakdasht region, Varamin, in South of Tehran during summer 2010. The experiment was carried out in plastic pot plantation

(18 × 15 cm) in a completely randomized design with 4 repetitions. Treatments included drought (at 3 levels, namely, no stress FC, moderate stress  $\frac{2}{3}$  FC and severe stress  $\frac{1}{3}$  FC) and antioxidant ascorbate (10 mM). The pots were filled with 4 kg sand-loam soil. Seeds of *Dracocephalum moldavica* L. plant were obtained from Pakan Bazr Company Isfahan, Iran. Plants were kept under natural conditions with the average temperature of 28°C. Drought stress was applied through measuring soil field capacity (FC) on 2 pots randomly selected from each treatment. In week 5, with imposing drought stress, ascorbate were also sprayed on the pots. The plants were then harvested in week 8.

**Measurement of Proline Content:** Proline content was measured using the method suggested by [17]. In this method, 0.1 g of fresh root and shoot tissues were separately homogenized in 10 ml of 3% sulphosalicylic acid. The solutions were then passed through filter paper. Then 1 ml of each solutions were mixed with 2 ml glacial acetic acid. The solutions were kept for 1 h in water bath and then immediately cooled down. Next, 4 ml toluene was added to each solution and after 30 minutes, its absorption in the wavelength 520 nm was measured. Finally, the amount of proline was determined in  $\mu\text{mol/L}$  from a standard curve.

**Measurement of Sugars:** In order to measure soluble and insoluble sugars, [18] method was used. Accordingly, 0.1 g root and shoot tissues were oven dried at 70°C for one week. The plant material was then mixed with 10 ml ethanol 80%. After one week, the solution on top of the vessel was used to measure soluble sugars content of the plants and the sediments were used for determining insoluble sugars. To determine soluble sugars contents, 2ml top layer solution was mixed with 1ml phenol 5% and then 5ml sulphuric acid was added to the solution. After 30 minutes, soluble sugars contents were spectrophotometrically determined by taking the absorbance at 485 nm. In order to determine insoluble sugars, the sediments were mixed with 50 cc water. The mixture was then boiled for 15 min at 100°C and then filtered through Watman No 2 filter paper. Two ml top layer from the solution was mixed with 1ml phenol 5% and then 5ml sulphuric acid was added to the solution. After 30 minutes, soluble sugars contents were determined by a spectrophotometer taking the absorbance at 485 nm. Glucose standard curve was used to determine both soluble and insoluble sugars concentration.

### Measuring Antioxidant Contents

**Determination of Flavonoids:** The method described by [19] was used to determine flavonoids contents. 0.1g of fresh tissues obtained from the extreme ends of roots and shoots was ground and centrifuged in 10 ml acidic ethanol (99:1, ethylic alcohol: acetic acid, respectively from left to right). The resulting solution was then warmed in a hot bath for 10 minutes and the absorbance at 270, 300 and 320 nm was recorded using the spectrophotometer. Flavonoids content was measured based on the absorbance percentage.

**Determination of Anthocyanins:** To determine anthocyanins contents of the plants, [20] method was used. First, 0.1 g fresh leaf from the tip of the shoots and root ends was ground and centrifuged in 10 ml acidic methanol (99:1 methylic alcohol: HCl respectively from left to right). The resulting solution was kept for one night in the dark and its absorbance was then measured with the spectrophotometer at 550 nm.

**Determination of Phenols:** The content of phenols was determined using the method described by [21]. 0.1 g fresh plant samples were obtained from roots and shoots. The samples were then boiled in 10 ml ethanol 80%. The samples were then centrifuged before they were added diluted Folin-Ciocalteu reagent and saturated sodium carbonate. Finally, phenol content was measured using the spectrophotometer at 640 nm against control apparatus. Catechol was used to draw the standard curve. Phenol contents were measured in mg/g fresh weight and expressed as catechol equivalents.

### Determination of Antioxidant Enzymes

#### To Measure These Enzymes, [22] Method Was Used

**Enzyme Extraction:** 1 g sample was mixed with 4ml extraction solution (1.2 g Tris, 2 g ascorbic acid, 38 g borax, 2 g EDTANA and 50 g polyethylene glycol). Distilled water was then added to make 100 ml solution. The solution was homogenized for 30 minutes and then kept at 4°C for 24 h before it was centrifuged at 400 g for half an hour.

**Determination of Peroxidase Enzyme Activity:** The peroxidase activity was determined by [22] method. Accordingly, 0.1 ml of enzyme extract was added to assay mixture containing 2 ml 0.2 M acetate buffers (pH 5.0), 0.4 ml of 3% H<sub>2</sub>O<sub>2</sub> and 0.2 ml of 0.01 M benzidin solution in 50% alcohol. The activity of enzyme was determined by

taking the absorbance of the reaction mixture at 530 nm. In order to protect enzyme activity, upper stages were done in ice dishes.

**Determination of Catalase Enzyme Activity:** The catalase activity was assayed by [23] method with the following modification: 5 ml of assay mixture for catalase activity contained 300 µM of phosphate buffer, (pH 6.8) 100 µM of H<sub>2</sub>O<sub>2</sub> and 1 ml of the twice diluted enzyme extracted. After incubation at 25°C for 1 min, the reaction was stopped by adding 10 ml of 2% (v/v) H<sub>2</sub>SO<sub>4</sub> and the residual H<sub>2</sub>O<sub>2</sub> was titrated against 0.01 N of KMnO<sub>4</sub> until a faint purple color persisted for at least 15 sec. One unit of catalase activity is defined as the amount of enzyme which breaks down 1 µ- M of H<sub>2</sub>O<sub>2</sub>/min under the described assay condition.

#### Determination of Ascorbate Peroxidase Enzyme Activity:

The method described by [24] was used to measure ascorbate peroxidase enzyme activity. 0.1 ml enzyme extract was solved in the reactive solution including 100 mmol potassium phosphate buffer (pH 7), 0.22 mmol ascorbate and 0.3 mmol H<sub>2</sub>O<sub>2</sub> 3%. The absorbance was then spectrophotometrically recorded at 290 nm. In order to protect enzyme activity, upper stages were done in ice dishes.

## RESULTS AND DISCUSSION

**Proline Content of Roots and Shoots:** As Fig. (1) shows, increase in drought stress resulted in increase in the proline content and the effect of ascorbate was significantly positive at  $P < 0.05$ . The highest and lowest proline level in shoots were observed under drought stress at  $\frac{1}{3}$  field capacity (FC) with 110.112 µmol/L ascorbate and no drought stress (19.232 µmol/L) respectively. The highest and lowest proline content in roots were recorded in the plants under drought stress at  $\frac{1}{3}$  FC with 112.43 µmol/L ascorbate and no drought stress (24.452 µmol/L) respectively. Under drought stress ( $\frac{1}{3}$ FC), even proline in roots was higher than in shoots.

Studies show that under drought stress, proline contents increases in shoots and particularly in roots. This was also the case of *Dracocephalum moldavica* L. in the present study where there was a rise in proline content especially in roots. This in turn led to a rise in osmotic pressure which stops roots and shoots from losing water. Ascorbate treatment improved the plants conditions and as a result, proline level decreased.

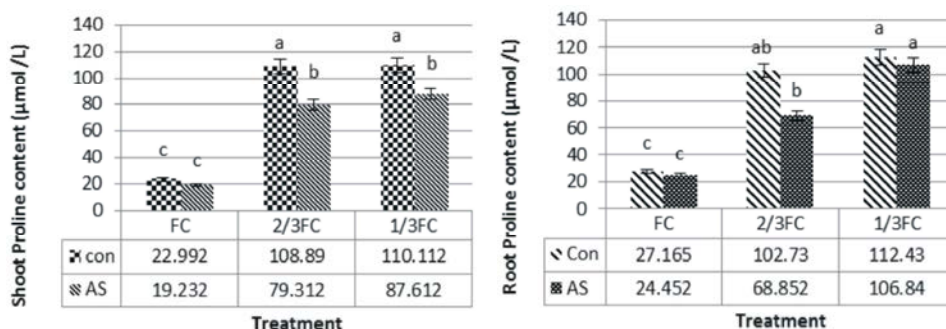


Fig. 1: Interaction of drought stress and ascorbate treatment on root and shoot proline contents. no drought stress (FC), mild stress (2/3 FC) and severe drought stress (1/3 FC); AS: ascorbate and Con: no ascorbat

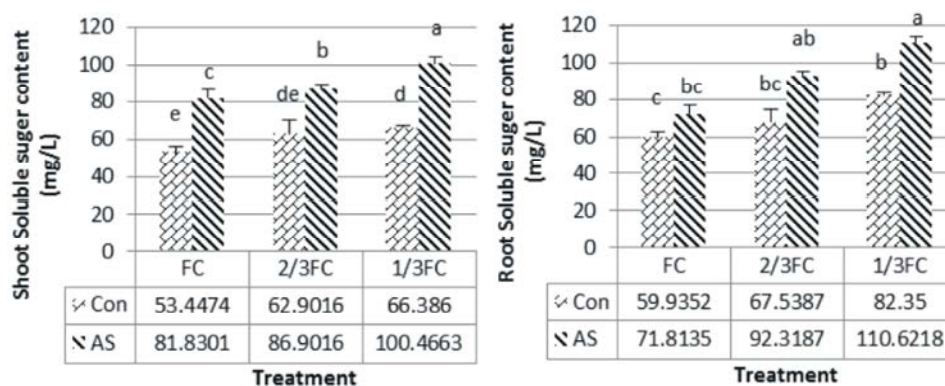


Fig. 2-1: Interaction of drought stress and ascorbate treatment on the contents of root and shoot soluble carbohydrates: no drought stress (FC), mild stress (2/3 FC) and severe drought stress (1/3 FC); AS: ascorbate and Con: no ascorbat

Increase in free proline has been reported in many cultivars under drought stress and this study is in line with findings of [25] on tomatoes and that of [26] who worked on maize. While application of ascorbate as an antioxidant [13] increased proline contents under various drought stress conditions, it reduced proline levels under normal conditions. This is similar to the findings of the study on okra reported by [13] a study reported by [27] who worked on halophytes and [28] who studied wheat.

**Soluble and Insoluble Sugars Content:** Various levels of drought stress reduced insoluble sugars in the plants under study (Figs. 2-1; 2-2) and the effect of ascorbate was significantly positive at  $P < 0.05$ . In shoots, the highest and lowest contents of insoluble sugars were recorded under no drought stress with 81.83  $\mu\text{mol/L}$  ascorbate (FC+As) and under severe drought stress ( $1/3$  FC) with 27.38  $\text{mgL}^{-1}$ . The highest and lowest contents of insoluble sugars in roots were recorded under no drought stress (FC+As) with 68.16  $\text{mg/L}$  ascorbate and severe drought stress 30.13  $\text{mg/L}$  (Figs. 2).

The study also showed that drought stress increased soluble sugars so that the resulting osmotic pressure could keep the plant from losing water. Although there is a cut in photosynthesis, the osmotic pressure occurs in cells as a result of decomposition of insoluble sugars. Therefore, imposing drought stress increases soluble sugars while it decreases insoluble sugars. Ascorbate treatment on the other hand, improves the plant conditions and increases photosynthesis. As a result, both soluble and insoluble sugars increase in the plants (Fig. 2).

The findings showed that ascorbate increased soluble sugars in control and various levels of drought stress. This however, decreased insoluble sugars in roots while increasing their level in shoots. In okra drought stress of sugars increases contents and applying ascorbate can increase photosynthesis and this, results in an increase of sugars. Accumulation of sugars in root helps the increase of osmotic reserves and as a result increases water absorption [13]. Starch is a carbohydrate that reduces during drought stress. This is probably a

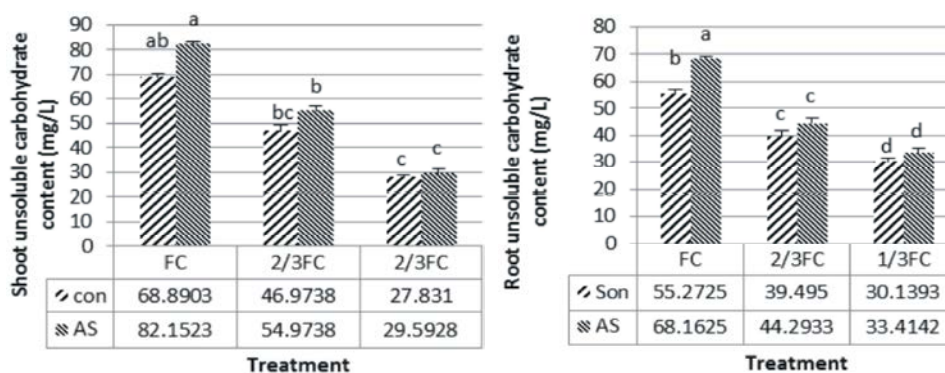


Fig. 2-2: Interaction of drought stress and ascorbate treatment on the contents of root and shoot insoluble carbohydrates: no drought stress (FC), mild stress (2/3 FC) and severe drought stress (1/3 FC); AS: ascorbate and Con: no ascorbat

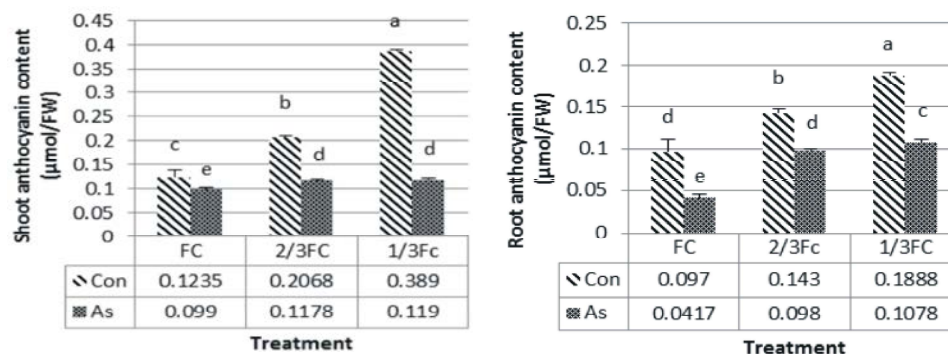


Fig. 3: Interaction of drought stress and ascorbate treatment on root and shoot anthocyanin contents: no drought stress (FC), mild stress (2/3 FC) and severe drought stress (1/3 FC); AS: ascorbate; and Con: no ascorbat

physiological response to water shortage. Respiration and growth in plants during low drought stress results in starch consumption. Consequently, carbohydrate supply drops. Also, soluble sugars in roots and shoots of plants under drought stress increases. Therefore, there seems to be a relationship between the decrease in starch content and soluble sugars accumulation. Accumulation of soluble sugars is also observed in a number of studies on other cultivars[29]. Drought stress in spinach leaves led to an increase in starch decomposition and accumulation of soluble sugars [30]. Thus, as leaf water potential decreases, sugar accumulation will probably play a key role in osmotic adjustment. It is also reported that in plants adapted to drought stress conditions, sugar accumulation may increase photosynthesis [29]. Soluble sugar contents in shoots and roots of the control and various levels of drought stress increased and this agrees with the findings of [5] in citrus and [38] in apple. On the other hand, simultaneous use of ascorbate improves the plant conditions. This is because they inactivate photosynthesis enzymes [6].

#### Antioxidant Activities

**Contents of Anthocyanins in Roots and Shoots:** Fig. 3 shows changes in anthocyanins contents under various levels of drought stress and their interaction with ascorbate in roots and shoots of *Dracocephalum moldavica* L. As the graph suggests, anthocyanins levels in shoots increased with severe drought stress (1/3 FC) so much that its concentration in shoots and roots reached  $0.11 \mu\text{mol gFW}^{-1}$  and  $0.05 \mu\text{mol gFW}^{-1}$  respectively. Findings suggest that ascorbate decreases anthocyanins contents both in roots and shoots.

**Contents of Flavonoids in Roots and Shoots:** Changes in the absorption percentage of flavonoids in shoots of *Dracocephalum moldavica* L. are shown in Fig. 4. Absorption percentage of flavonoids in shoots increased up to 0.18 % under severe drought stress ( $1/3$  FC). Application of ascorbate reduced flavonoids at various drought stress levels. As Fig. 4 suggests, drought stress and its interaction with ascorbate have a meaningful effect on flavonoids absorption. Flavonoids absorption in roots

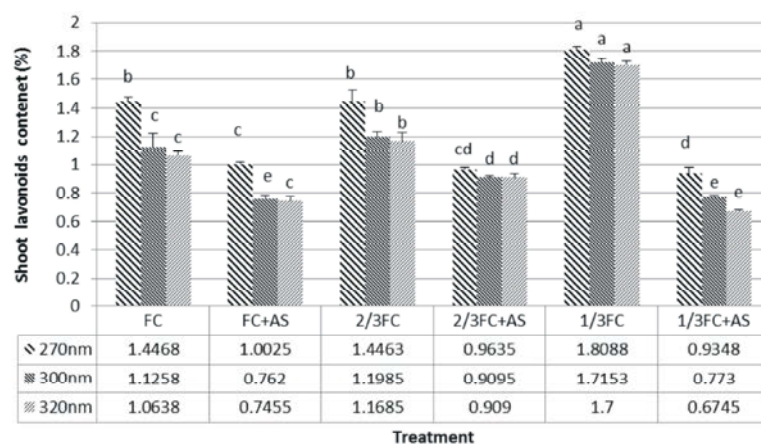


Fig. 4-1: Interaction of drought stress and ascorbate treatment on shoot flavonoid contents: no drought stress (FC), mild stress (2/3 FC) and severe drought stress (1/3 FC); AS: ascorbate; and Con: no ascorbat

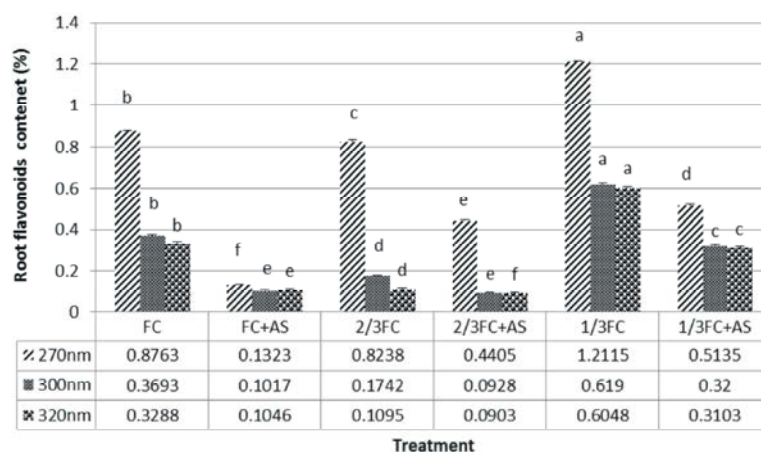


Fig. 4-2: Interaction of drought stress and ascorbate treatment on root flavonoid contents: no drought stress (FC), mild stress (2/3 FC) and severe drought stress (1/3 FC); AS: ascorbate and Con: no ascorbat

increased under severe drought stress ( $1/3$  FC). Moreover, ascorbate reduced flavonoids absorption at various levels of drought stress.

**Phenolic Compounds in Roots and Shoots:** Fig. 5 shows variations in phenolic compounds of *Dracocephalum moldavica* L. under drought stress and their interaction with ascorbate. As the graph suggests, imposing drought stress increased phenolic compounds levels in roots and shoots both with ascorbate ( $1/3$  FC + AS) and without ascorbate ( $2/3$  FC). The increases for shoots and roots were  $64.81$  ( $\text{mg gFW}^{-1}$ ) and ( $67.85$   $\text{mg gFW}^{-1}$ ), respectively.

**Root and Shoot Peroxidase Enzyme:** Variations in peroxidase activities in *Dracocephalum moldavica* L. under drought stress and their interaction with ascorbate are shown in Fig. 6. Peroxidase activity in shoots and

roots increased under severe drought stress ( $1/3$  FC) as it was  $1.79$  ( $\mu\text{mol H}_2\text{O}_2/\text{min}$ ) and  $1.64$  ( $\mu\text{mol H}_2\text{O}_2/\text{min}$ ) in shoots and roots, respectively. Also ascorbate treatment increased peroxidase activity. This improved the plants conditions and their resistance against activated oxygen free radicals produced under drought stress.

**Root and Shoot Catalase Enzyme:** Fig. 7 shows variations in catalase activities in the plants under drought stress and its interaction with ascorbate. Catalase activities in roots and shoots increased under severe drought stress ( $1/3$  FC) as they were  $0.50$  ( $\mu\text{mol H}_2\text{O}_2/\text{min}$ ) and  $0.55$  ( $\mu\text{mol H}_2\text{O}_2/\text{min}$ ) in shoots and roots, respectively. Moreover, ascorbate increased catalase activities and this improved the plants conditions, increasing their resistance against active oxygen radicals produced under drought stress.



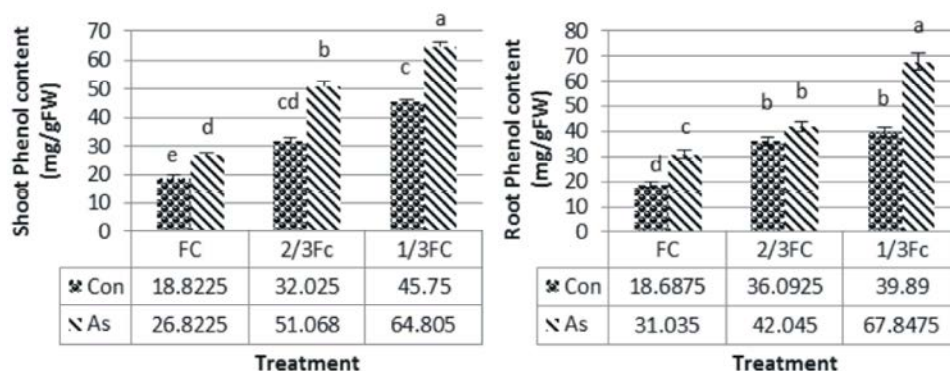


Fig. 5: Interaction of drought stress and ascorbate treatment on root and shoot phenolic compound contents: no drought stress (FC), mild stress (2/3 FC) and severe drought stress (1/3 FC); AS: ascorbate and Con: no ascorbat

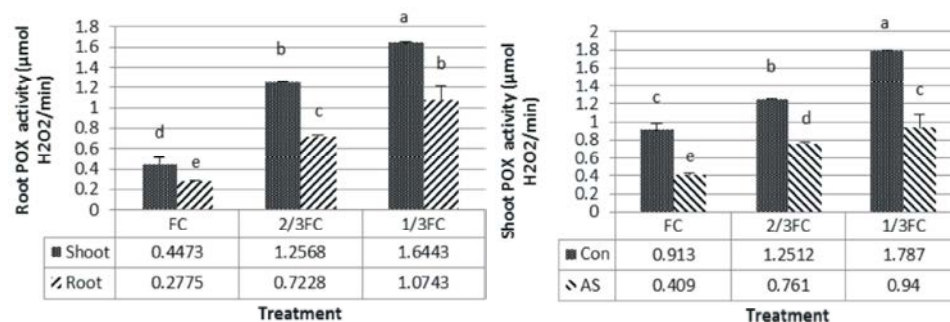


Fig. 6: Interaction of drought stress and ascorbate treatment on peroxidase activities in roots and shoots: no drought stress (FC), mild stress (2/3 FC) and severe drought stress (1/3 FC); AS: ascorbate and Con: no ascorbat

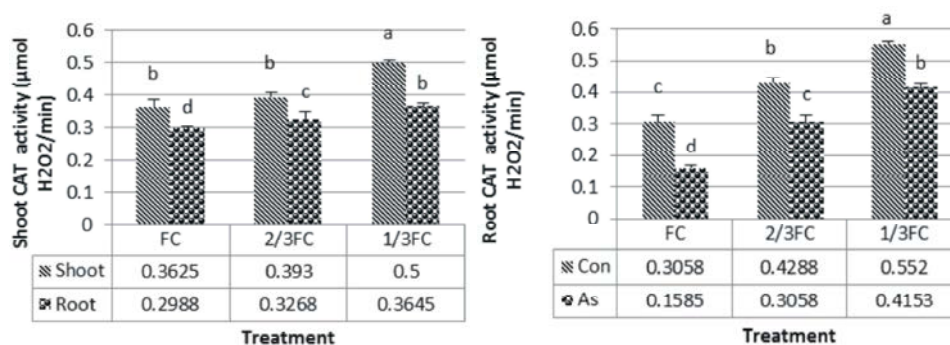


Fig. 7: Interaction of drought stress and ascorbate treatment on catalase activities in shoots: no drought stress (FC), mild stress (2/3 FC) and severe drought stress (1/3 FC); AS: ascorbate and Con: no ascorbat

**Root and Shoot Ascorbate Peroxidase Enzyme:** Fig. 8 presents ascorbate peroxidase activities in *Dracocephalum moldavica* L. under drought stress and their interaction with ascorbate. As the graph shows, ascorbate peroxidase increased in roots and shoots under severe drought stress (1/3 FC) as it was recorded 0.56 (μmol H<sub>2</sub>O<sub>2</sub>/min) and 0.51 (μmol H<sub>2</sub>O<sub>2</sub>/min) in shoots and roots, respectively. Again ascorbate treatment increased the enzyme activities and improved the plants conditions by increasing their resistance against active oxygen radicals produced under drought stress.

A better resistance against drought coincides with significant rise in antioxidants activities. However, it is not clear if this is because of an increase in synthesis of these enzymes or increase in their activities. Increase in transcription of genes responsible for synthesis of various stress metabolites, including antioxidant enzymes, is reported in resistant cultivars in response to stress [30]. This study showed that applying drought stress increased antioxidant enzymes (catalase, peroxidase and ascorbate peroxidase) and ascorbate improved helped improve plant conditions. Numerous studies indicate that,

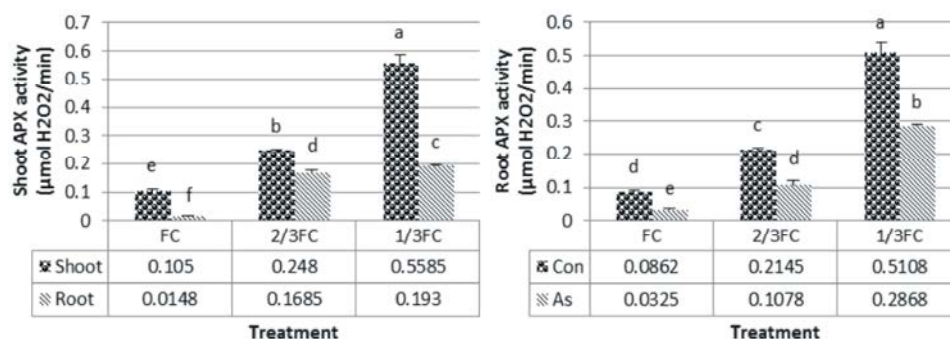


Fig. 8: Interaction of drought stress and ascorbate treatment on ascorbate peroxidase activities in shoots: no drought stress (FC), mild stress (2/3 FC) and severe drought stress (1/3 FC); AS: ascorbate and Con: no ascorbat

under stress, changes occur in oxidation and reduction systems within mitochondria. This leads to production of ROS which play a role in plant defense mechanisms and communications. ROS accumulation in plant cells is a reaction to external environmental factors; however, due to peroxidation in lipid membrane and collapse of DNA strands they also destroy cells [32].

Ascorbate treatment improved this enzyme performance. In fact, the best catalase performance in shoots of *Dracocephalum moldavica* L. was recorded under  $\frac{1}{3}$  FC and ascorbate application. [33] also reported increased catalase under drought stress. They contended that the highest catalase activity in drought resistant varieties is an indication of its better capability to decompose hydrogen peroxide. The same researchers also showed that there existed a positive correlation between drought stress and increased catalase activity in drought resistant varieties of cotton [33].

Application of drought stress to *D. moldavica* showed that with increase in drought stress, peroxidase activity in roots and shoots increased and this increase was more pronounced after application of ascorbate. This indicates the significance of ascorbate in regulation of peroxidase activity in oxidative stress due to drought. In drought stressed *D. moldavica*, peroxidase activity in all treatments showed a meaningful increase and ascorbate led to a better performance of this enzyme in all levels of drought stress. Also, this enzyme was more active in roots than in shoots. Considering the general consensus among researchers about a strong relationship between the increase in peroxidase activity and drought stress resistance and in view of the finding of the present study about ever more increase in peroxidase activity in drought stressed *D. moldavica*, one can perceive the resistance of this plants to drought stress. Increase in peroxidase activity was also reported in cotton under drought stress [30]. Many evidences suggest that

peroxidase related reactions play an important role in aging as peroxidase activities increases with age; therefore, a part of peroxidase activity can be attributed to the aging process in plants [30]. Peroxidase activity in roots and shoots of *D. moldavica* increased in all treatments with and without presence of ascorbate; however, under no drought stress condition (control), ascorbate increased and decreased peroxidase activity in shoots and roots respectively. There was a rise in peroxidase activity under various levels of drought stress when ascorbate was applied. Generally, this enzyme was more active in shoots than in roots. The rise in activity of antioxidant enzymes such as peroxidase reduces  $O_2$ ,  $H_2O_2$  concentrations and through this, reduces the risk of hydroxyl radical [15]. External application of ascorbate activates antioxidant mechanisms and this improves resistance in the plants drought stress [11].

Some metabolites such as carotenoids, flavonoids, anthocyanins and ascorbic acid sweep free radicals protecting the plant against oxidative stresses. Carotenoids are able to absorb high levels of energy from low wavelength and release ROS and through absorbing radicals from generated oxygen play their antioxidant role. The cell membrane and other internal membranes (chloroplast and mitochondria membranes) are composed of two phospho-lipid layers. Superoxidated radicals produced under drought stress cause the peroxidation of lipids [34]. Flavonoids and anthocyanins in leaves act as the recipient of free radicals and protect the plant against oxidative stresses [10].

Phenol compounds, flavonoids and anthocyanins in plants mainly possess antibacterial qualities [13]. Many antibacterial effects in plant extracts are because of tannins, phenol compounds and similar chemicals that exist in different plant parts such as roots, leaves, seedlings, plantlets and skins [35]. Phenol compounds exist in many plants and the present research studied their



total content in *D. moldavica*. Their antibacterial effect depends on the position and number of hydroxyl groups on phenolic loop and a direct relationship is claimed to exist between the hydroxyl groups of phenols and their toxic effect on microorganisms [13]. Moreover, oxidated phenols have a more severe effect. Possible mechanism in these compounds is enzymatic control through reaction with sulfidril or non-specialized reaction with bacterial proteins [13]. Also, flavonoids because of their oxidation role prevent oxidation either directly through engaging in reduction reactions or indirectly through chelating ferrous [36]. The present study showed that in all drought stress treatments, phenol compounds, anthocyanins and flavonoids increased in roots and shoots of *D. moldavica*. On the other hand, when ascorbate was applied, phenol compounds increased while there was a reduction in flavonoids and anthocyanins contents. External application of ascorbic acid activated antioxidant mechanism and thus made the plant resistant to drought stress [13], ascorbate 10 mM treatment helping sweep ROS reduced anthocyanins and flavonoids and this was in agreement with findings of [37], on green beans under salt stress and supplemented with gibberellin and also with [8] who worked on *Withania somnifera* under stress.

Finally it could be concluded that under no drought stress, ascorbate application in *D. moldavica* reduces anthocyanins, flavonoids, phenol compounds, peroxidase, catalase and ascorbate peroxidase activities. Under drought stress, antioxidants, phenol compounds, anthocyanins and flavonoids activities increases and ascorbate application reduces anthocyanins and flavonoids while it increases antioxidant enzymes and phenol compounds.

#### ACKNOWLEDGEMENTS

I thank Mahlagh Ghorbanli for many stimulating discussions on the importance of time scale in the consideration of regulatory mechanisms and Maryam peyvandy for perceptive and helpful comments on the manuscript.

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