

## **Effect of Salicylic Acid Application on Oxidative Damage and Antioxidant Activity of Grape (*Vitis vinifera* L.) Under Drought Stress Condition**

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(Received: 10 February 2016, Accepted: 13 April 2017)

### **Abstract**

Adaptation and defense responses are the consequences of exposure to drought stress in plants. Salicylic acid (SA) as a natural signaling molecule poses a significant impacts on various aspects of plant growth and development. This study investigates the effects of SA on carotenoids content, lipid peroxidation rate and antioxidant enzymes activities. A pot experiment was conducted using a factorial pattern based on a randomized complete block design with three replications under greenhouse conditions. The variables in the experiment included grapevine cultivars ('Rasheh' and 'Bidane Sefid'), irrigation periods (5, 10 and 15 day intervals) and SA concentrations (0, 1 and 2 mM). Results showed that with increasing the drought stress levels, increase in carotenoids, malondialdehyde (MDA) contents, catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) enzymes activities were detected. Carotenoids content, catalase (CAT) and ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) enzymes activities were higher in the Rasheh cultivar compared with the 'Bidane Sefid' cultivar. Carotenoids content, CAT, APX and GPX enzymes activities increased by application of salicylic acid. According to the obtained results, the Rasha cultivar showed a greater tolerance to water stress in comparison with Bidane Sefid cultivar, suggesting that SA is capable of inducing drought stress tolerance in plants.

**Keywords:** Antioxidant enzymes, Drought stress, Grapevine, Malondialdehyde, Salicylic acid.

### **Introduction**

Based on various observations, water stress induces oxidative stress in plants (Manivannan et al., 2008). Reactive Oxygen Species (ROS) including superoxides ( $O_2^{\cdot-}$ ); hydroxyls ( $OH^{\cdot}$ ); perhydroxyls ( $HO_2^{\cdot}$ ) and, alkoxyls ( $RO^{\cdot}$ ) beside non-radical (molecular) forms [hydrogen peroxide

( $H_2O_2$ ) and singlet oxygen ( $O_2$ )] (Gill and Tuteja, 2010), adversely affect the proteins, lipids, carbohydrates, and nucleic acids (Bian and Jiang, 2009). Intensity of damage caused by ROS depends on the balance between the level of ROS and their degradation rate by antioxidant scavenging mechanisms (Azooz et al., 2009). On the other hand, it has been reported that the membranes of plant cells can be affected by the rapid damage

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following exposure to water stress. Uncontrolled enhancement of free radicals which cause lipid peroxidation can lead to leakage of the membrane. Damage to fatty acids of membrane produces small hydrocarbon fragments such as malondialdehyde (MDA) (Moussa and Aziz, 2008). Furthermore, MDA as a final product of plant cell membrane lipid peroxidation represent the membrane system injury (Cunhua et al., 2010). During evolution, plants have developed unique defense mechanisms to increase their tolerance under adverse conditions which help them to survive under such unfavorable growth conditions (Xu et al., 2008). To do so, plants alternatively synthesize the antioxidant enzymes involved in defense mechanisms against different stresses.. The increased activity of antioxidant enzymes including catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) consequently result in delay of leaf senescence. However under severe drought stress condition, damages caused by the active oxygen to the cells are not effectively reduced by antioxidant machinery.. Therefore, a wide range of compounds are utilized for decreasing the adverse impacts of stress on crops. SA is an endogenous growth regulator with phenolic nature and also an important signaling molecule which regulates physiological processes in plants such as photosynthesis and some other metabolic processes (Syed et al., 2011; Nazar et al., 2011; Khan et al., 2012). The significant role of SA in modulating the plant response to various abiotic stresses is supported by many studies (Hayat et al., 2008; Kadioglu et al., 2011). In addition, it has been observed that plant exposure to SA generally caused a higher resistance against drought stress (Al-Hakimi and Hamada, 2001; Hayat et al., 2008; Kadioglu et al., 2011). SA has been shown to be capable of generating a wide range of metabolic responses. Furthermore SA role in plant-water relation has been proven (Hayat et al. 2010). It has been observed that drought-stressed plants

supplemented with exogenous SA are effective in modulating both enzymatic and non-enzymatic components of antioxidant defense system (Wang et al., 2004; Kadioglu et al., 2011). The aim of the present work was to investigate the effect of SA treatment on morphological and physiological characteristics in two grapevine cultivars (Rasha and Bidanese fid) under drought stress.

## Materials and Methods

Two- years old grape saplings were planted in plastic pots (20 cm in diameter, 24 cm in depth and 7 li capacity) containing 6 Kg soil, including clay, sandy loam soil and sand in a ratio of 1:2:1 respectively. The experiment was conducted during the 2012 March- 2013 August season (6 month) at Research green house in the Faculty of Agriculture, Urmia University in Iran. Temperatures in the green house were adjusted on  $40 \pm 2$  °C during day and  $17 \pm 2$  °C at night, with relative humidity of ~45% and a photoperiod of 14 h. The experiment was carried out as a factorial based on randomized complete block design with three factors including irrigation periods (every 5, 10 and 15 days), SA concentrations (0, 1 and 2 mM) and grape cultivars (Rasha and Bidanese fid) with three replications.

The spraying of SA was conducted when the first irrigation cycle started (3 month after seedling culture and adaption to greenhouse condition for 90 day). In addition, to prevent SA toxic effect, the next sprayings were done every 15 days interval.

The height of the seedlings is measured at the beginning and end of the experiment and the longitudinal growth ratio of the seedlings at the beginning and the end of the experiment was measured using a ruler and ultimately the height difference was calculated. The following formula was used to create a uniformity between the comparisons (Cramer et al., 1986).

Longitudinal growth ratio = Secondary longitudinal growth - Primary longitudinal growth / Primary longitudinal growth  $\times 100$

In order to measuring leaf area, at the end of the experiment three leaves (small, medium, big) from each sapling were collected and the leaf area was measured by Leaf Area Meter (Model AM200) and was multiplied by number of all leaves in every correspond sample. The leaf thickness and length of roots were measured at the end of the experiment by ruler and caliper respectively.

In order to measure the number of stomata, samples were prepared from the lower part of the leaf and from a similar place of the leaves with same size using covering the leaves by colorless nail polish. The dried layer of nail polish was removed and then checked by microscope and the numbers of stomata from eight similar points were counted and their average was calculated.

Relative water content (RWC) was calculated by the following equation (Filella et al., 1998):

$$\text{RWC (\%)} = (\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight}) \times 100$$

At the end of the experiment, Lipid peroxidation was determined by measuring Malondialdehyde (MDA) content (Heath & Packer 1968). Fresh leaf samples (0.2 g) were homogenized in 5 ml of 1% trichloroacetic acid (TCA) and centrifuged at 8000 g (model, company, city and country) (NüveFüj 650 model centrifuge) at 1,500 rpm for 10 min. One ml of supernatant was mixed with 4 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA and the mixture was incubated in boiling water for 30 min and transferred to an ice bath to stop the further reaction. The absorbance was read at 532 nm using an Analytik Jena Specord 200 model spectrophotometer and adjusted for nonspecific absorbance to 600 nm. MDA content was estimated by using an extinction coefficient of  $155 \text{ mM}^{-1} \text{ cm}^{-1}$ .

The Chlorophyll a, b and carotenoid content was determined according to Lichtenthaler and Wellburn (1983). The weighed fresh leaf samples were extracted with 100% acetone and were homogenized with the B-Brawn type homogenizer at 1000 rpm for one minute. The homogenate was filtered by two layer cheese cloths and was centrifuged using the NüveFüj 650 model centrifuge at 1500 rpm for ten minutes. The supernatant was separated and the absorbance of acetone extracts was measured at 662 nm, 645 nm and 470 nm using an Analytik Jena Specord 200 model spectrophotometer. The Chlorophyll a, b and total content of carotenoids were calculated from the equations mentioned below (Lichtenthaler and Wellburn, 1983).

$$\text{Chl a} = 11.75 \times A_{662} - 2.35 \times A_{645}$$

$$\text{Chl b} = 18.61 \times A_{645} - 3.96 \times A_{662}$$

$$\text{Car} = 1,000 \times A_{470} - 2.27 \times \text{Chl a} - 81.4 \times \text{Chl b} / 227$$

Soluble protein content was estimated by the method of Bradford (1976) and Bovine serum albumin (BSA) was used as a standard.

Catalase activity ( $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1} \text{ FW}$ ) was assayed by measuring the initial rate of hydrogen peroxide disappearance (Aebi, 1984). The reaction mixture contained 2.5 ml of 50 mM potassium phosphate buffer (pH 7.4), 0.2 ml of 1% hydrogen peroxide and 0.3 ml of enzyme extract. The homogenate was centrifuged at 15000 g for 15 min at 4 °C and the supernatant was immediately used for the enzyme assay. Decrease in hydrogen peroxide was measured as the decline in absorbance at 240 nm and the activity was calculated using the extinction coefficient of  $43.6 \text{ mM}^{-1} \text{ cm}^{-1}$  for hydrogen peroxide.

ascorbate peroxidase (APX) activity was determined by the method of Asada (1992). Samples (fresh leaf) of 0.2 g were homogenized in 1 mL of 50 mM Na-phosphate buffer (pH 7.8) containing 0.1 mM ADTA, 1mM Na-ascorbate, 0.2 ml of 10 mM hydrogen peroxide and 0.1 ml of

enzyme extract. The reaction was initiated by adding H<sub>2</sub>O<sub>2</sub> to the solution. The decrease in absorbance was monitored at 290 nm.

Activity of guaiacol peroxidase (GPX) was determined in a reaction mixture consisted of 1 ml of 1% guaiacol, 1 ml of 1% H<sub>2</sub>O<sub>2</sub>, 2.5 ml of 50mM Na-phosphate buffer (pH=7) and 0.1 ml enzyme extract. Activity was determined by the increase of absorbance at 470 nm due to guaiacol oxidation ( $E= 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ ) (Updhyaya et al., 1985). Statistical analysis was carried out using SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA) and Comparing averages were carried out by one-way ANOVA using Duncan test.

## Results

### *Plant Height*

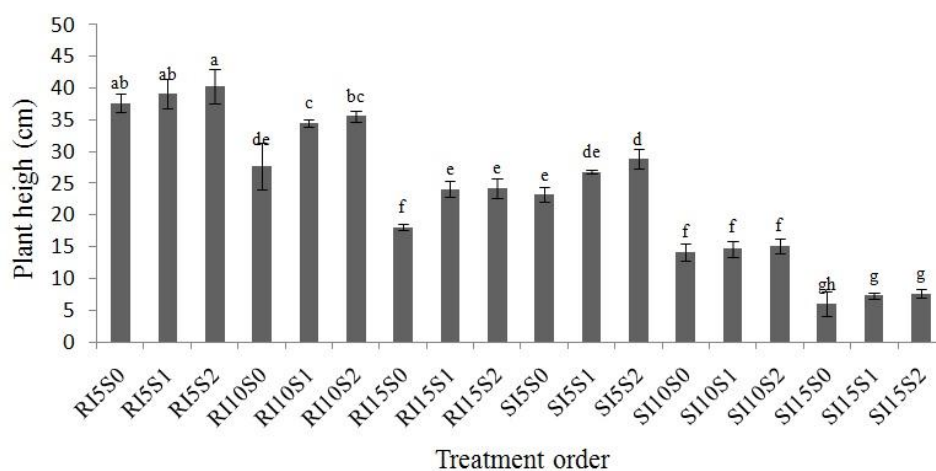
The results of data analysis, revealed a significant effect of drought stress levels, salicylic acid, variety, and interaction of varieties with drought and SA on plant height. The results of comparing averages showed that by increasing drought, the plant height in both varieties, Rasha and Bidanese fid, decreased. The plant height in Rasha was more than Bidanese fid and SA application with 2 mM concentration in irrigation cycles of every 10 and 15 days increased plant height in both varieties. Based on the obtained results the maximum plant height was observed in Rasha with watering cycle of every 5 days containing 1 and 2 mM concentration of SA and the minimum plant height was observed in Bidanese fid with watering cycle of every 15 days and 0 mM SA (Fig. 1).

### *Leaf properties (number, area, thickness), number of stomata and root length*

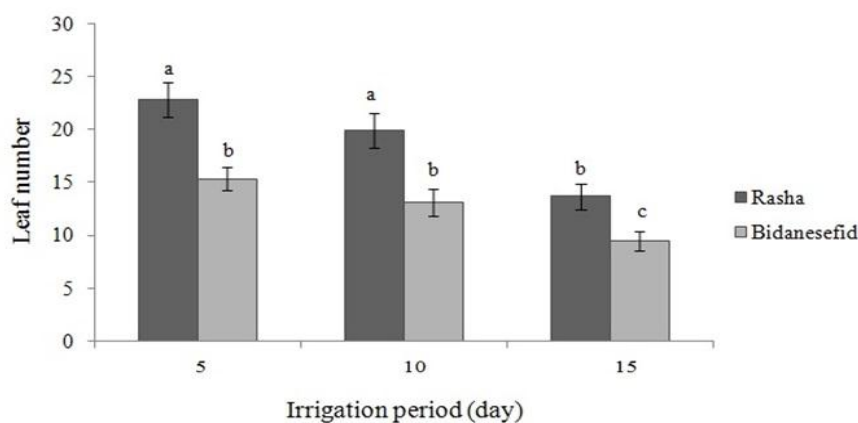
Analysis of variance showed that the effect of variety, drought stress levels, SA

treatment, interaction of drought with SA and interaction of variety with drought on both leaf number and area were significant. However, increasing the severity of drought reduced the leaf number and leaf area formed on treated plants of both varieties. Comparing the averages showed that by increasing irrigation intervals, leaf number and leaf area significantly decreased. However, the number of leaves in Rasha was higher than Bidanese fid, leaf area in Bidanese fid was more than Rasha (Fig. 2, 4). SA application increased the leaf number and leaf area, and 2 mM concentration of SA had the most effect.. The results showed that the maximum and minimum leaf number and leaf area was respectively observed in every 5 days irrigation regime (control) with 2 mM concentration of SA and those with every 15 days irrigation cycle with 0 mM concentration of SA respectively (Fig. 3, 5).

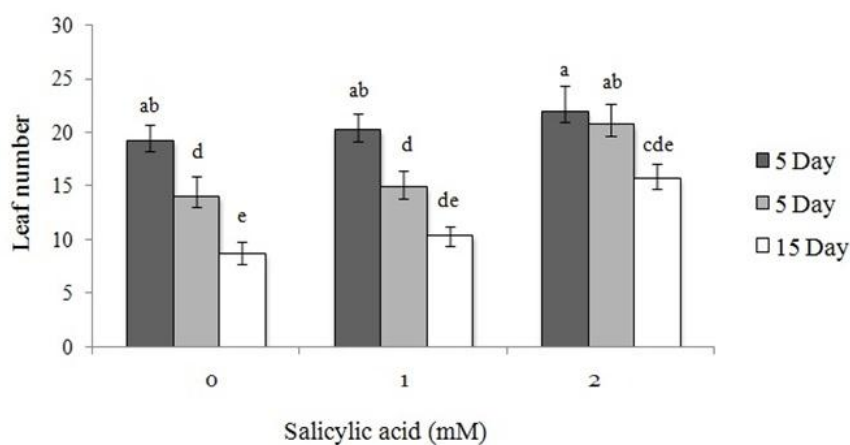
Also, the effect of SA treatment and interaction of variety and drought on stomata number and triple interaction of variety, drought and SA on leaf thickness were significant. The results of comparing averages showed that by increasing drought intensity, the number of stomata and leaf thickness in both varieties increased. However this increase was higher in Rasha compare to the Bidane sefid cultivar (Fig. 6, 7). Application of 2 mM SA concentration in irrigation cycles of once every 10 and 15 days increased leaf thickness and number of stomata in both varieties. Results showed that in Rasha, the maximum and minimum leaf thickness was respectively observed in irrigation cycle of once every 15 days with 2 mM concentration of SA and once every 5 days with 0 mM concentration of SA (Fig 6).



**Fig. 1.** Interaction of variety, drought and salicylic acid (SA) on plant heigh. R: Rasha cv., S: Bidanesefid cv., S0: SA0 mM (control), S1: SA 1 mM, S2: SA 2 mM, I5: Irrigation cycle of 5 days (control), I10: Irrigation cycle of 10 days, I15: Irrigation cycle of 15 days. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE (n=18).



**Fig. 2.** Interaction of various levels of drought and variety on leaf number. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE (n=6).



**Fig. 3.** Interaction of various levels of drought and Salicylic acid on leaf number. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE (n=9).

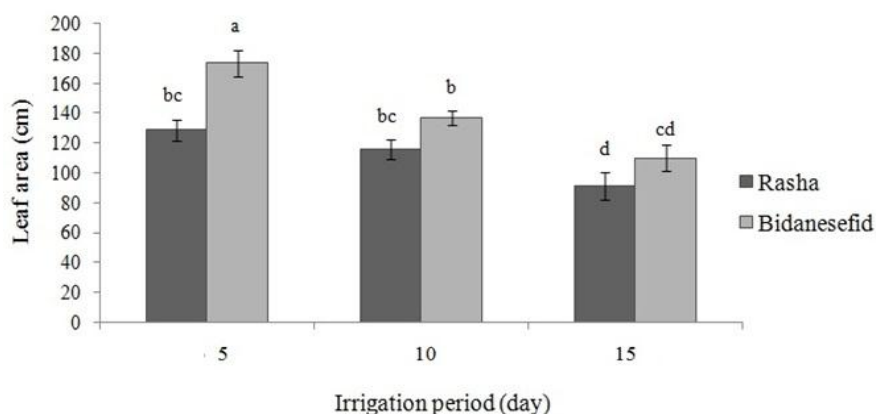


Fig. 4. Interaction of various levels of drought and variety on leaf area. Similar letter/s shows not significant differences. ( $P < 0.01$ ). Error bars show mean  $\pm$  SE (n=6).

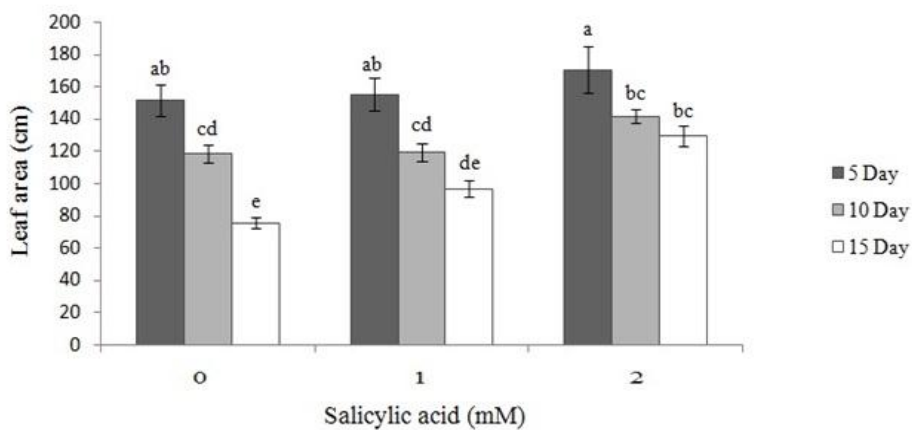


Fig. 5. Interaction of various levels of drought and Salicylic acid on leaf area. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE (n=9).

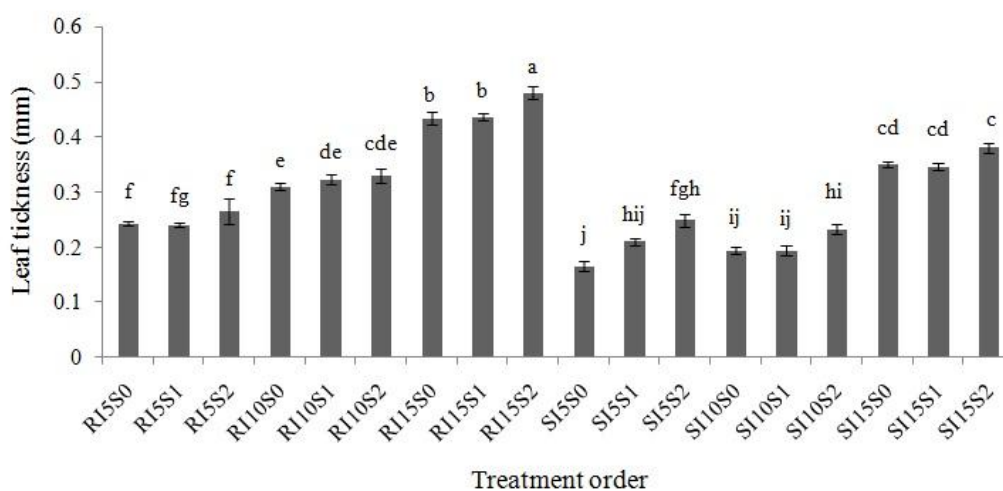


Fig. 6. Interaction of variety, drought and salicylic acid (SA) on Leaf Thickness. R: Rasha cv., S: Bidanesefid cv., S0: SA 0 mM (control), S1: SA 1 mM, S2: SA 2 mM, I5: Irrigation cycle of 5 days (control), I10: Irrigation cycle of 10 days, I15: Irrigation cycle of 15 days. Similar letter/s shows not significant differences. ( $P < 0.01$ ). Error bars show mean  $\pm$  SE (n=18).

The maximum numbers of stomata in Rasha was with irrigation cycle of once every 15 days and the minimum numbers of stomata in Bidanese fid was observed in watering cycle of 5 days (Fig. 7). SA application in different levels was associated with increase in leaf area and resulted in the decrease of the stomata number (density per unit area). However, concentration of 2 mM SA showed the highest effect (Fig. 8).

Also, Based on the analysis of variance results, variety and drought stress levels showed the significant effect on root length. Comparing averages showed that, by increasing drought intensity the root length increased and the root length in Bidane sefid was more than Rasha cultivar (Fig. 9, 10).

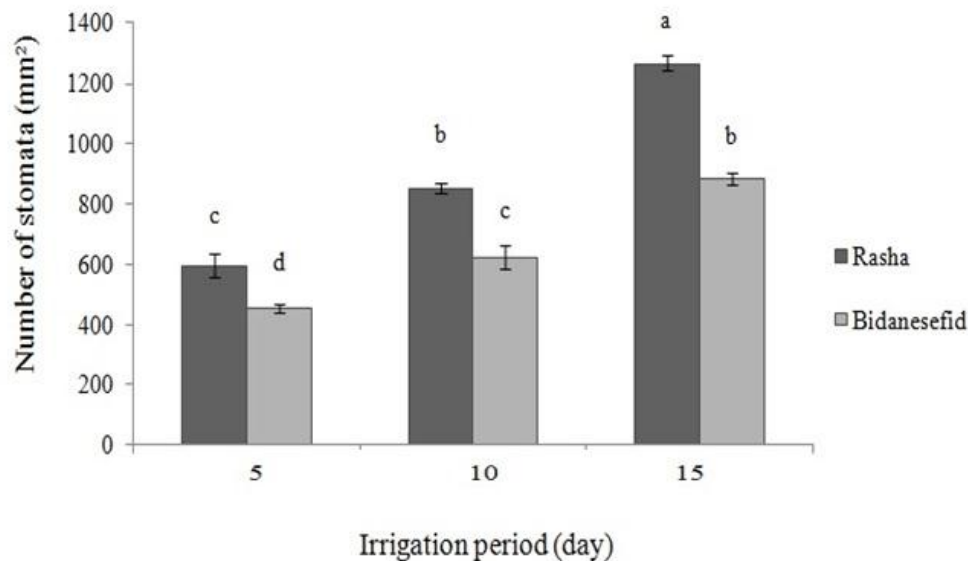


Fig. 7. Interaction of various levels of drought and variety on number of stomata. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE ( $n=6$ ).

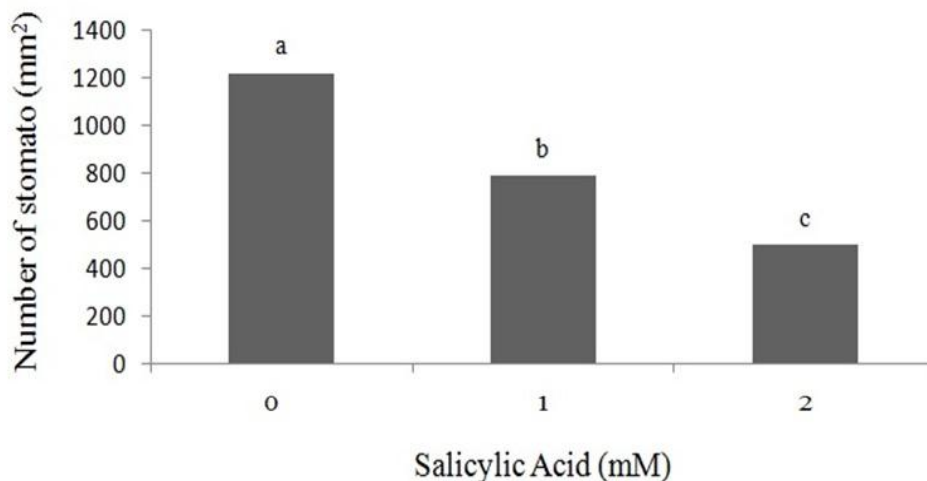


Fig.8. Effect of salicylic acid levels on number of stomata. Similar letter/s shows not significant differences ( $P < 0.01$ ).

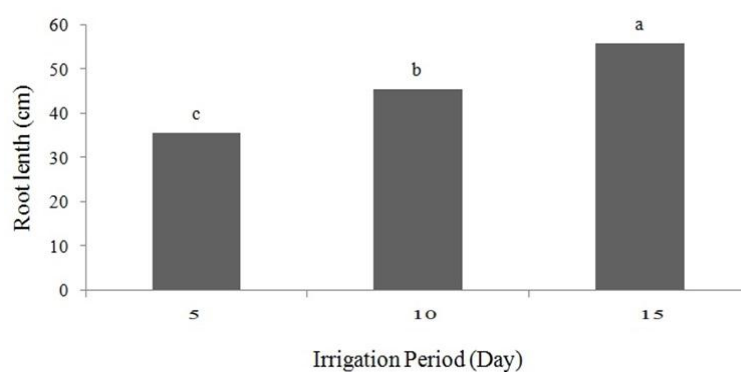


Fig. 9. Effect of various levels of drought on root length. Similar letter/s shows not significant differences ( $P < 0.01$ ).

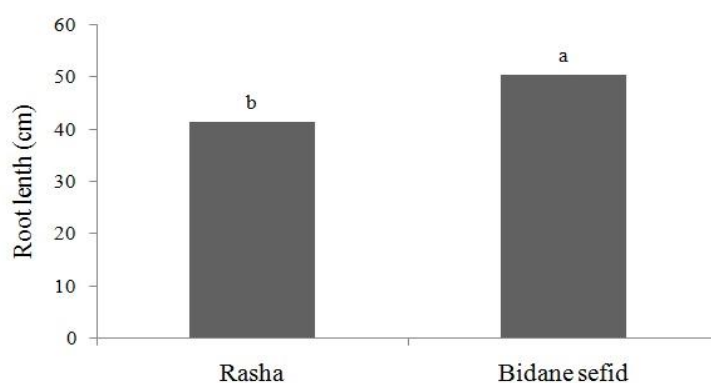


Fig. 10. Effect of variety type on root length. Similar letter/s shows not significant differences ( $P < 0.01$ ).

### ***Relative water content (RWC)***

There was a significant effect of various levels of drought and variety on leaf RWC ( $P < 0.01$ ). Based on the results of comparing averages, the RWC was significantly reduced under water stress and significantly increased at recovery stage comparing to the

water deficit conditions in both cultivars. Maximum relative content of leaf water was observed in 5 days and the minimum amount was occurred in irrigation cycle of 15 days (Fig. 11). Moreover, the results showed that RWC was higher in Rasha when compared to the RWC of Bidane sefid (Fig. 12).

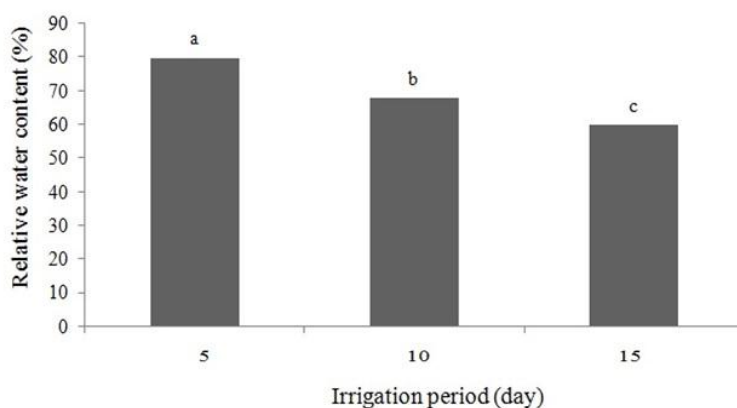


Fig.11. Effect of various levels of drought on Relative water content (RWC). Similar letter/s shows not significant differences ( $P < 0.01$ ).



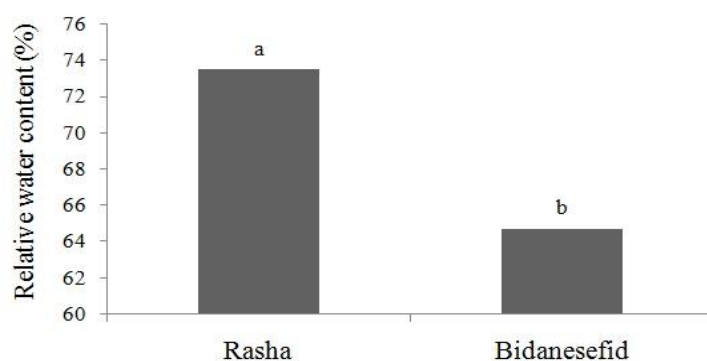


Fig.12. Effect of variety type on Relative water content (RWC). Similar letter/s shows not significant differences ( $P < 0.01$ ).

### Chlorophyll a, b and Carotenoids

Interaction of drought and SA and 3-way interactions of drought level, SA and variety on chlorophyll a content ( $P < 0.01$ ) were significant. Moreover, the results provided from comparing the mean squares related to interactions of variety, drought and SA on chlorophyll a showed that the amount of chlorophyll a in Rasha was higher than Bidane sefid. However, SA treatment with concentration of 2 mM increased the chlorophyll a content (Fig. 13).

Analysis of variance revealed a significant effect of 3-way interactions of drought level, SA and variety on chlorophyll a and interaction of variety and different levels of drought on chlorophyll b and carotenoids contents. Analysis of

interaction between variety and different levels of drought on chlorophyll b and carotenoids showed that by increasing of irrigation intervals a significant increase in carotenoids content and decrease in chlorophyll b were occurred. The highest and lowest chlorophyll b and carotenoid contents were observed in Rasha with 5 and 10 days and in Bidane Sefid with 15 days irrigation intervals, respectively (Fig. 13, 14, 15). On the other hand, salicylic acid application decreased the harmful effects of drought stress. Salicylic acid application resulted in the increase of carotenoids and 2 mM salicylic acid had the most effect on the carotenoids accumulation (Fig. 16).

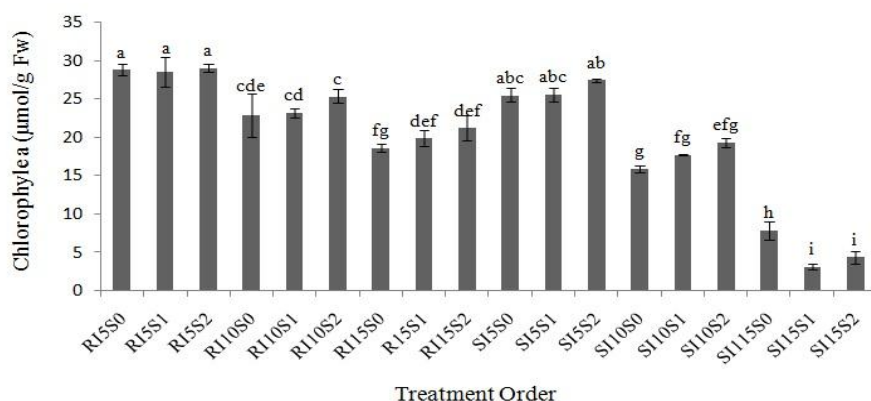


Fig. 13. Interaction of variety, drought and salicylic acid (SA) on chlorophyll a. R: Rasha cv., S: Bidanesefid cv., S0: SA 0 mM (control), S1: SA 1 mM, S2: SA 2 mM, I5: Irrigation cycle of 5 days (control), I10: Irrigation cycle of 10 days, I15: Irrigation cycle of 15 days. Similar letter/s shows not significant differences. ( $P < 0.01$ ). Error bars show mean  $\pm$  SE ( $n=18$ ).

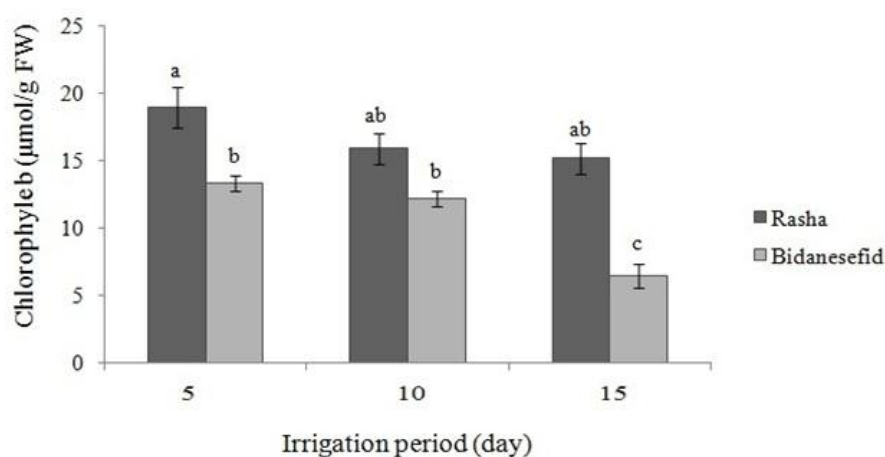


Fig. 14. Interaction of various levels of drought and variety on chlorophyll b. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE (n=6)

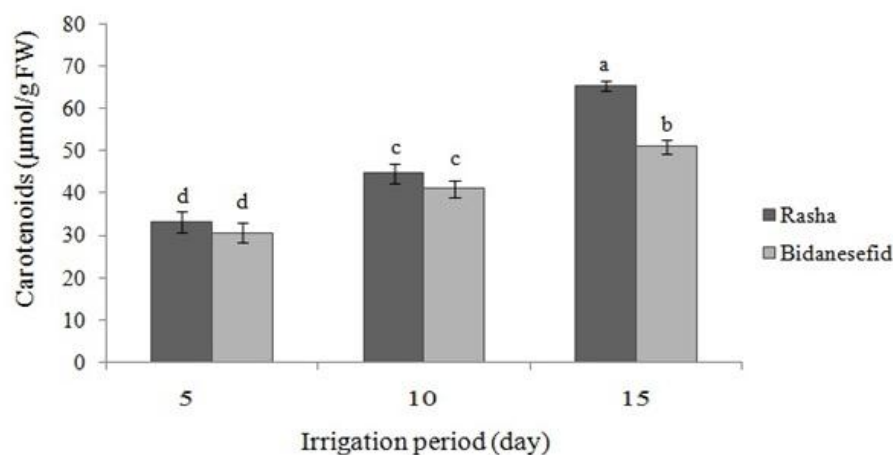


Fig. 15. Interaction of various levels of drought and variety on carotenoids. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE (n=6).

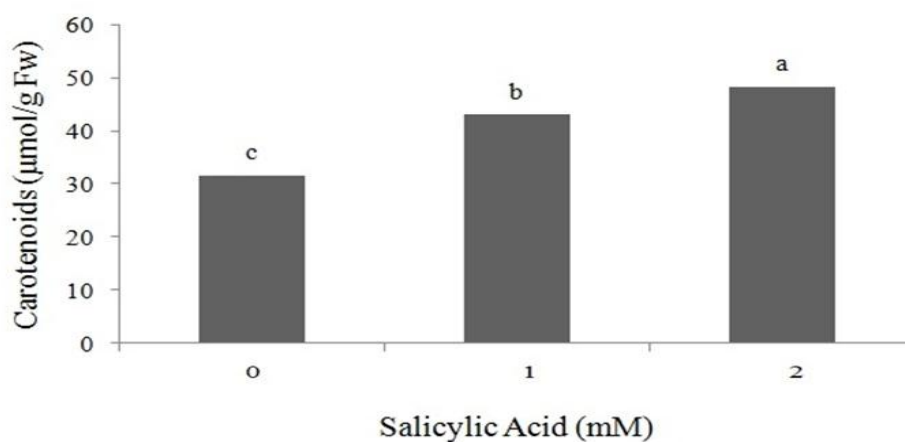
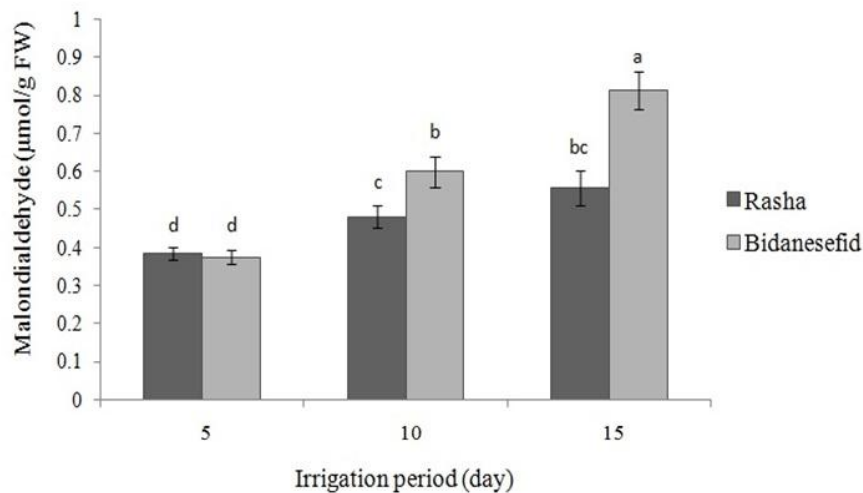


Fig.16. Effect of Salicylic Acid levels on carotenoids. Similar letter/s shows not significant differences ( $P < 0.01$ ).

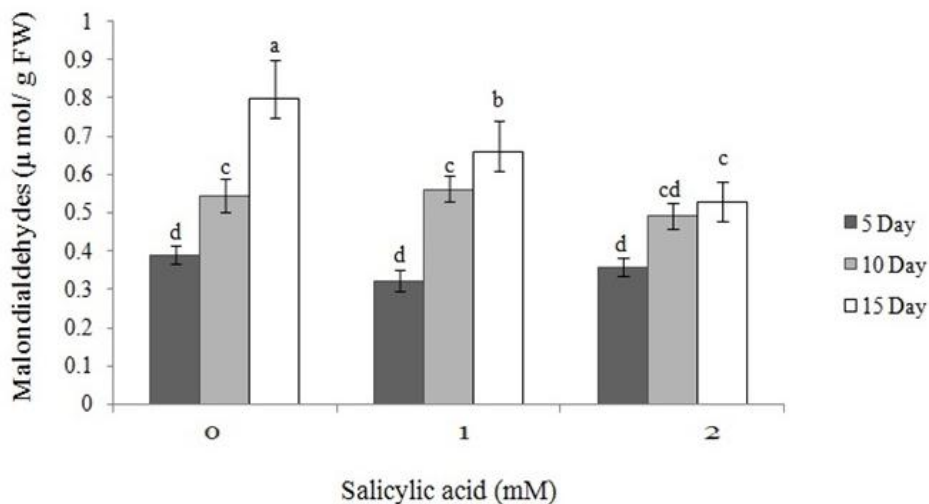
### ***Malondialdehyde (MDA) and total protein***

Comparing the averages related to interaction of various drought extents and Salicylic acid, showed that drought stress significantly raised the MDA content and total protein when compared to the control plants (Fig. 17 and 19). However, MDA level in Bidane Sefid was higher than Rasha while the total protein in Rasha was more

than that of Bidane Sefid. Plants treated with SA (2 mM) showed the minimum MDA content. Furthermore plants irrigated every 5 days with 0, 1 and 2 mM SA level had lower MDA contents in comparison with other irrigation strategies (Fig. 18). Maximum total protein was detected when irrigation was done every 15 days with 2 mM SA concentration (Fig. 20).



**Fig. 17.** Interaction of various levels of drought and variety on malondialdehyde. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE (n=6).



**Fig. 18.** Interaction of various levels of drought and Salicylic acid on the malondialdehyde. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE (n=9).

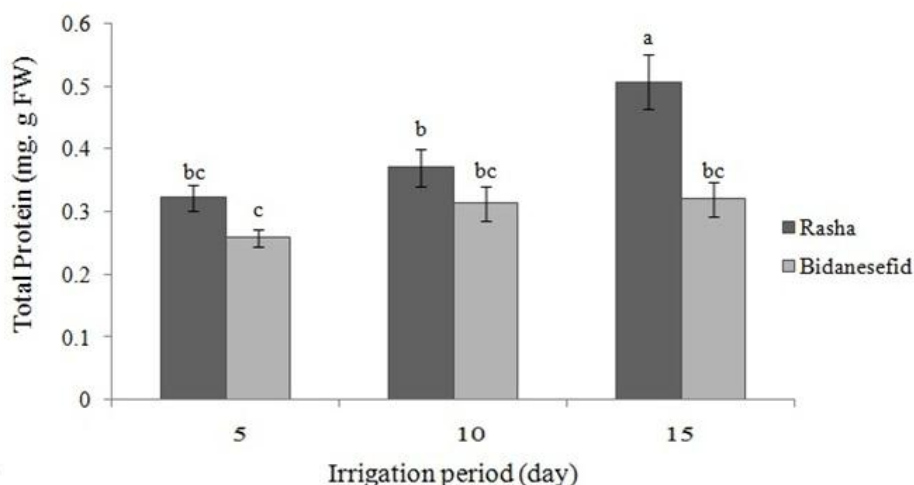


Fig. 19. Interaction of various levels of drought and variety on total protein. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE ( $n=6$ ).

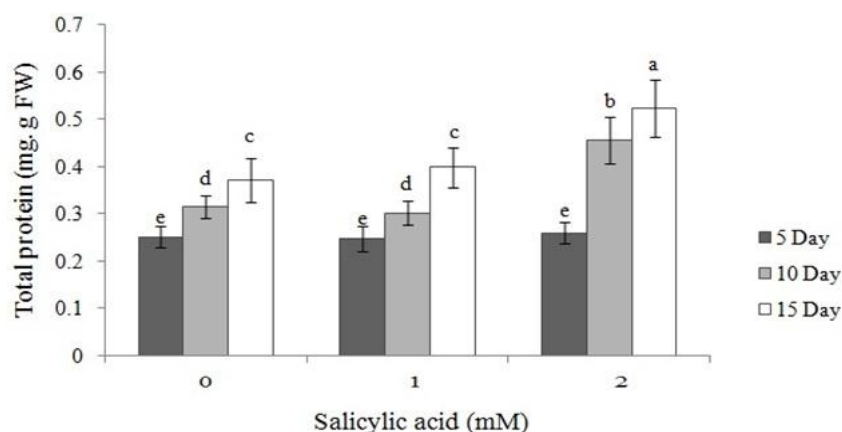


Fig. 20. Interaction of various levels of drought and salicylic acid on total protein. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE ( $n=9$ ).

### ***Antioxidant enzymes***

According to the results of various analyses, there were significant interactions between drought and SA and 3-way interactions of drought level, SA and the variety observed in the amounts of catalase (CAT) and guaiacol peroxidase (GPX) activities. Moreover, the effect of variety, drought stress levels and SA treatment on ascorbate peroxidase (APX) activities were significant ( $P < 0.01$ ). According to the results obtained from comparing the mean square values related to interactions of varieties, drought stress induced an increase in catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase

(GPX) activities and antioxidant enzymes activity of Rasha was higher than Bidanesefid (Fig. 21, 22, 24, 25). Antioxidant enzymes activities were enhanced with increase in the SA concentration. As shown in Figures 21, 23 and 25, SA treatments increased the activity of catalase (CAT) and guaiacol peroxidase (GPX) and ascorbate peroxidase (APX) in both cultivars. The maximum amount of enzyme (CAT, GPX, APX) activities were observed in Rasha when irrigation regime was every 15 days, and salicylic acid applied with 2 mM concentration.

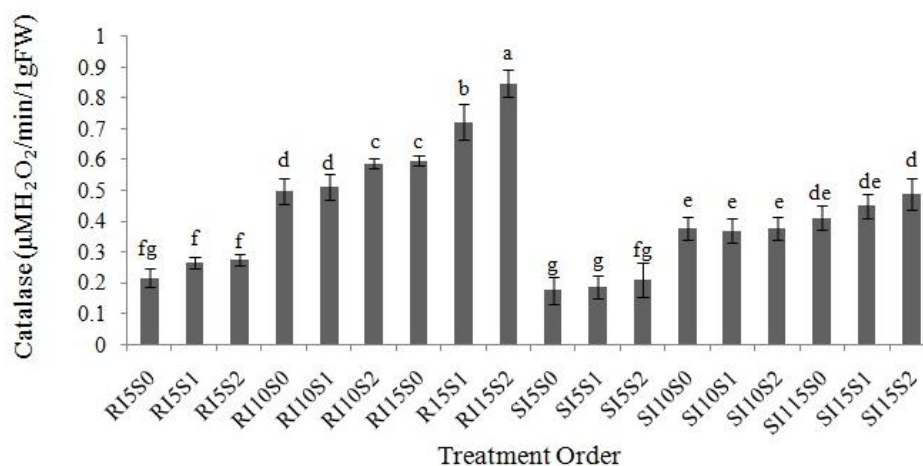


Fig. 21. Interaction of variety, drought and salicylic acid (SA) on (CAT) catalase activities. R: Rasha cv., S: Bidanesefid cv., S0: SA 0 mM (control), S1: SA 1 mM, S2: SA 2 mM, I5: Irrigation cycle of 5 days (control), I10: Irrigation cycle of 10 days, I15: Irrigation cycle of 15 days. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE ( $n=9$ ).

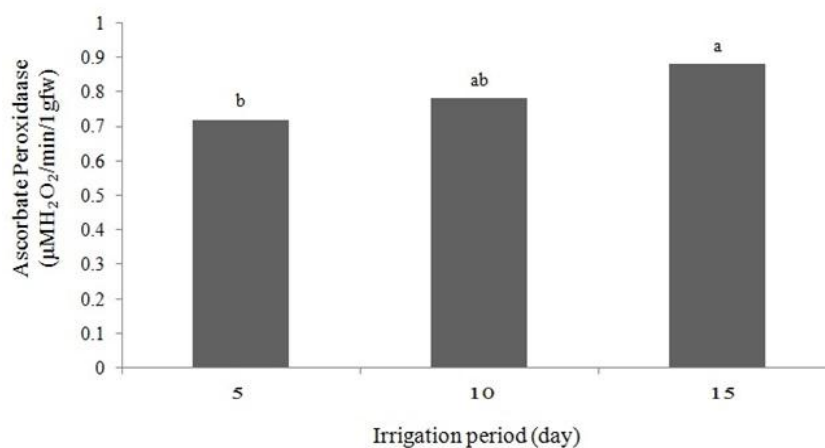


Fig. 22. Effect of various drought levels on ascorbate peroxidase (APX) activities. Similar letter/s shows not significant differences ( $P < 0.01$ ).

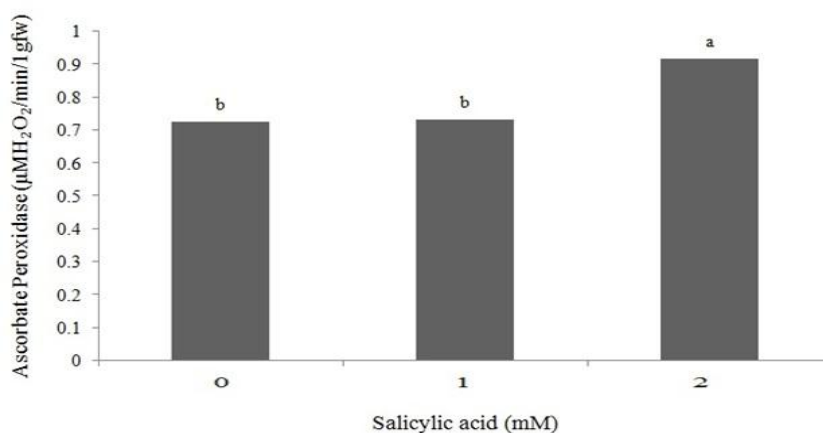


Fig. 23. Effect of salicylic acid levels on ascorbate peroxidase (APX) activities. Similar letter/s shows not significant differences ( $P < 0.01$ ).

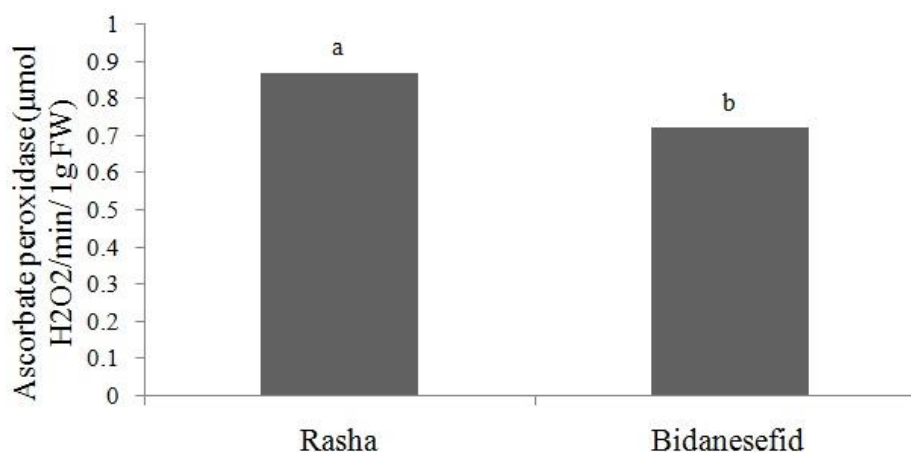


Fig.24. Effect of variety type on ascorbate peroxidase (APX) activities. Similar letter/s shows not significant differences ( $P < 0.01$ )

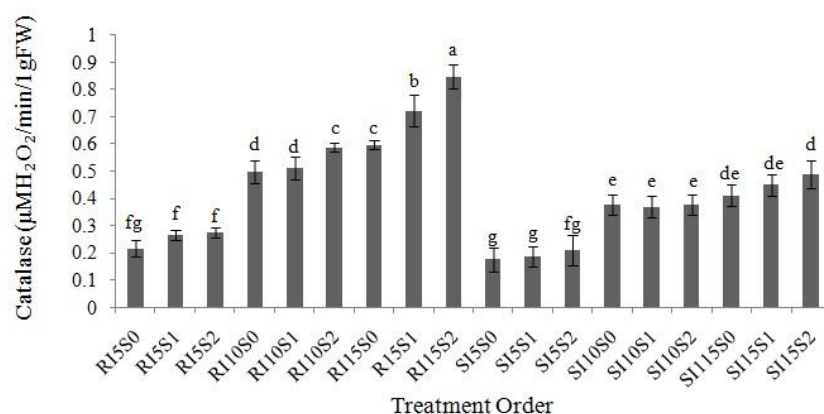


Fig. 25. Effect of variety, drought and salicylic acid (SA) on guaiacol peroxidase (GPX) activities. R: Rasha cv., S: Bidanesefid cv., S0: SA 0 mM (control), S1: SA 1 mM, S2: SA 2 mM, I5: Irrigation cycle of 5 days (control), I10: Irrigation cycle of 10 days, I15: Irrigation cycle of 15 days. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE (n=9).

## Discussion

### Plant Height

Cell growth is one of the most drought-sensitive physiological processes due to the reduction of cell turgor pressure (Taiz and Zeiger, 2006). Under severe water deficiency, cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells (Nonami, 1998). Impaired mitosis, cell elongation and expansion result in reduced plant height, leaf area and crop growth under drought stress (Kaya et al., 2006; Hussain et al., 2008). It is

confirmed that SA reduces accumulation and leakage of toxic ions in plants (Zhou et al, 2009) and decreases the effects of environmental stresses by increasing the number of growth regulating hormones such as auxins and cytokines (Shakirova et al, 2003). Sadeghipour and Aghaei (2002) found that plant height of common bean was decreased by the drought pressure and application of SA enhanced plant growth. Umebese et al (2009) reported that the positive effect of SA on tomato stem height was due to the capability of this compound to stimulate antioxidant activity that protect plant from perilous effects of drought

pressure and develop mitosis and cell elongation. Beside, Maity and Bera (2009) and Khan et al (2003) stated that the affirmative effect of SA in mung bean (*Vigna radiata*) was because of the SA effects in enhancement of biochemical and physiological processes or induction of N, P, K and Ca accumulation and antioxidant enzyme activity and glutathione accumulation.

### ***Leaf properties (number, area, thickness), number of stomata and root length***

There is an inverse relationship between increasing the severity of drought and leaf number and area of both varieties. By increasing irrigation intervals, leaf number and area significantly decreased in both cultivars. Such reduction in leaf number and area due to water stress can be attributed to its direct effect on cell division which arise from reduction in nucleic acid synthesis and/or enhancement of its breakdown (Ashraf et al, 1996). The reduction in leaf number in response to stress can also be attributed to enhancement of leaf abscission due to hormonal imbalance which arise from increased abscisic acid and decreased indole acetic acid (IAA) levels in treated plants (Arora et al, 2001). Previous reports indicated that drought tolerant species reduce the water loss either by reducing the leaf area or restricting stomatal opening or both (Lazaridou et al., 2003; Abbate et al., 2004; Lazaridou and Koutroubas, 2004). Agamy et al (2013), Zamaninejad et al (2013), and Sure et al (2011) reported that application of SA increased number of leaves under stress conditions. This positive effect of SA could be related to increasing of CO<sub>2</sub> assimilation and photosynthetic functionality and increasing of mineral uptake in stressed plant under SA application (Szepesi et al., 2005). It has been reported that application of SA and other relative analogues to the leaves, increase leaf area in corn and soybean

plants (Khan et al., 2003). Similar effect was obtained by Cornelia et al (2010) under stress conditions. Gharib (2006) and Khan et al (2003) reported that application of SA enhanced photosynthesis rate and increased the leaf area..

In dry conditions, the fluffs over the leaves epidermis have lower growth. Also leaf area has lower growth and the numbers of stomata per leaf area unit increases. Moreover, in dry conditions leaves create stronger mechanical tissues. The created morphological changes because of dryness are called Xeromorphs and these changes include: decreasing of leaf area, thickening of cell membrane and cuticle, and decreasing of cells growth (Jalili marandi et al., 2011).

Roots are the key plant organ for adaptation to drought stress conditions. If tolerance is defined as the ability to maintain leaf area and growth under prolonged vegetative stage stress, the main basis of variation could be attributed to the constitutive root system architecture that allows the maintenance of more favorable plant water status (Nguyen et al., 1997). Root size and thick root system allows plant to access more water in the soil, which was considered as an important identity in determining drought resistance in upland rice (Kavar et al., 2007).

### ***Relative water content (RWC)***

Relative water content (RWC), leaf water potential, stomatal resistance, transpiration rate, leaf temperature and canopy temperature are important characteristics that influence plant-water status. Relative water content reflects the metabolic activity in tissues and used as a strong index for dehydration tolerance. RWC of leaves is higher in the initial stages of leaf development and declines when the dry matter accumulates and leaf matures. RWC varies water uptake in the roots and water loss by transpiration in the leaves. A decrease in the RWC in response to drought stress has been reported in wide

variety of plants. Nayyar and Gupta (2006) reported that when leaves are subjected to drought, large reductions in RWC and water potential occur. Exposure of plants to drought stress substantially decreased the leaf water potential, relative water content and transpiration rate, with a concomitant increase in leaf temperature (Siddique et al., 2001). Various studies have shown that when leaves are subjected to drought, they exhibit large reductions in relative water content and water potential (Decov et al., 2000; Efeoglu et al., 2009). Moreover, it is known that dehydration is often reversible.

### ***chlorophyll a, b and Carotenoids***

Ethylene is considered as a stress related factor and induces many antioxidant responses and metabolites through activation of certain enzymes such as lipoxygenase (induction of chlorophyll catabolism), catalase, peroxidase (induction of chlorophyll catabolism and lipid peroxidation), cellulase (degradation cell wall) and polygalacturonase (Chae et al., 2003; Sage et al., 1989). Furthermore, metabolites such as carotenoids, flavonoids, anthocyanins and ascorbic acid protect the plant against oxidative damages by scavenging reactive oxygen species (ROS) (Sairam et al., 1998; Woodson et al., 1998). The highest and lowest chlorophyll b and carotenoid content was observed in Rasha with 5 and 10 and in Bidanese fid with 15 days irrigation intervals, respectively (Fig. 13, 14, 15). Furthermore, SA application decreased the negative effects of drought stress. SA (2 mM) caused the strongest effect on the increase of carotenoids (Fig. 16). The efficiency of exogenous SA is affected by the species, developmental stages of the plant, the SA application form and its concentration (Horvath et al, 1973; Joseph et al, 2010). SA caused the activation of the synthesis of carotenoids, xanthophylls and the rate of de-epoxidation but decreased the level of chlorophyll pigments, in wheat plants (Moharekar et

al., 2003); SA also increased the concentration of chlorophyll and carotenoid in maize plant (Khodary, 2004). Enhancement of photosynthetic capacity as a result of SA application can be attributed to the stimulatory effects on Rubisco activity and pigment contents. Application of SA in proper concentrations may temporarily induce an oxidative stress in plants, which is able to improve the antioxidative capacity of the plants and help to induce the synthesis of protective compounds such as carotenoids (Hayat and Ahmad, 2007). SA causes an enhancement in the level of chlorophyll and carotenoid pigments, photosynthetic rate, carboxylase activity of rubisco, and modification of the activity of the important enzymes (Hayat and Ahmad, 2007).

### ***Malondialdehyde (MDA) and total protein***

Superoxide radicals are often produced during drought stress and cause lipids peroxidation (Sairam et al, 1998; Sarkar and Tophan, 1974). Membrane lipid peroxidation leads to the production of compounds such as malondialdehyde (MDA), propanol, botanal, hexanal, heptanal and propanal dimethylacetal. These compounds are considered as an index for measuring the amount of peroxidation. Beside, increased level of the lipids peroxidation was considered as an index for increased oxidative stress conditions (Heat and Pacher, 1969; Meirs et al., 1992). In this study, the amount of MDA decreased following by SA application. Determination of MDA content among other parameters was used in confirming the protective effect of salicylic acid. Various studies have investigated the effect of SA on the ACC synthase and ACC oxidase (Zhu, 2001). Increasing the activity of antioxidant enzymes such as CAT, POD and SOD by SA ultimately leads to the ability of plants to protect plants against ROS generation and lipid peroxidation (Hayat et al., 2010).



According to the results of previous studies, susceptible plants represent higher levels of MDA and electrolyte leakage compared to those plants with higher tolerance (Juan et al., 2005). In previous studies, plants pretreated with SA exhibited a reduction in the level of lipid peroxidation and leakage of electrolytes from plant tissues as well as more intensive growth processes when compared to the control plants (Hayat and Ahmad, 2007). Plant resistance mechanisms are not known clearly however, the accumulation of new proteins along with and the expression stress related genes coding biosynthetic enzymes in response to the osmotic stress were investigated (Vallivodan and Nguyen, 2006). Increased levels of osmotic proteins were observed in mild water stress condition (Hajheidari et al., 2005). The roles of late embryogenesis abundant (LEA) proteins have not been identified completely, however some evidence indicates that they affect by increasing of plant resistance against drought stress which ultimately induce their production (Wise and Tunnacliffe, 2004). As shown in figure 20, SA treatment increased protein contents under drought conditions. SA caused high levels of protein content in soybean (Kumar et al., 1999) and wheat (Singh and Usha, 2003). Increasing the activation of nitrate reductase and nitrate contents leads to the increase in protein content of plants treated with SA (Fariduddin et al., 2003). The plants treated with SA also showed a considerable nitrate reductase activity, thereby results in maintaining the level of diverse proteins in their leaves (Singh and Usha, 2003). According to Mohammadkhani and Heidari (2008), the initial increase in total soluble proteins during drought stress is attributed to the expression of new stress proteins. This suggest that SA exerts its effect on regulation of the hormonal functioning through contribution of the protective reactions in plants, acceleration

of reparative processes and the effect on protein contents (Hayat and Ahmad, 2007).

### ***Antioxidant enzymes***

In our experiments significant interactions between drought and SA application and 3-way interactions of drought level, SA application and the variety were observed for catalase (CAT) and guaiacol peroxidase (GPX) activities. Moreover the effect of variety, drought stress levels and SA treatment on ascorbate peroxidase (APX) activities were significant ( $P < 0.01$ ). According to the results obtained from comparing the mean square values related to interactions of varieties, drought stress induced an increase in catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) activities. Antioxidant enzymes activity of Rasha was higher than that of Bidane Sefid (Fig. 21, 22, 24, 25). Antioxidant enzymes activities were enhanced with the increase in the SA concentration. As shown in figures 21, 23 and 25, SA treatments increased the activity of catalase (CAT) and guaiacol peroxidase (GPX) and ascorbate peroxidase (APX) in both cultivars. The maximum amount of these enzymes (CAT, GPX, APX) activity was observed in Rasha when irrigation was done every 15 days and SA treatment with 2 mM concentration. Increase in CAT, APX and GPX activities in leaves is most probably due to the increased level of ROS, and particularly  $H_2O_2$ , under water stress condition (Smirnoff, 1993). Active oxygen species, which are produced by plants under oxidative stress damage plant growth due to their detrimental effects on the sub cellular components and metabolism. In this regard superoxide ( $O_2^{\cdot-}$ ) radical is generated because of delaying the dark reactions of photosynthesis under environmental stresses. Therefore the excessive light energy cannot be used for the reduction of  $NADP^+$  in the ferredoxin of the chloroplast (Asada and Takahashi, 1987). Results

obtained from previous studies indicate that the higher concentrations of catalase and ascorbate peroxidase might contribute to the removal of the  $O_2^{\cdot-}$  radicals and its products such as  $H_2O_2$  which is induced under water stress conditions. Excessive production of reactive oxygen species in the photosynthetic apparatus under water stress conditions results in an increase of antioxidant content. In general, reduced irrigation interval causes an increase in the concentration of antioxidant enzymes, suggesting that the production of antioxidant enzymes can be a common response in plants under drought stress conditions (Farooq et al., 2009). Results from numerous studies have determined a correlation between the antioxidant enzymes activity and plant tolerance to the abiotic stresses, such as responses to drought stress in wheat (Shao et al., 2007; Abdullah and Ghamdi, 2009; Hasheminasab et al., 2012), alfalfa (Wang et al., 2009), rice (Sharma and Dubey, 2005; Qin et al., 2010), chickpea (Mohammadi et al., 2011), and ornamental plants such as marigold (Sedghi et al., 2012). SA increases the activity of antioxidant enzymes such as CAT, POD and SOD which in turn protect plants against ROS generation and lipid peroxidation (Hayat et al., 2010). SA treatment also provided a considerable protection against nitrate reductase leading to maintaining the level of diverse proteins in leaves (Sing and Usha, 2003). Mohammadkhani and Heidari (2008) found that the initial increase in total soluble proteins during drought stress was due to the expression of new stress proteins. The results obtained from these studies indicate that high and moderate dosages of SA enhance the antioxidant status and induce resistance under stress condition (Yalpani et al., 1994). Studying on the application of SA showed that this compound is capable of plants resistance induction under drought stress (Senaratna et al., 2000).

## Conclusion

Our findings indicate that under similar irrigation cycles in both varieties, grape would utilize different mechanisms to cope with drought stress conditions. Our findings showed that by increasing irrigation intervals, the amount of carotenoids, total protein and APX, CAT, GPX enzymes activity were significantly enhanced in Rasha cultivar, and that these parameters were higher than that of Bidane sefid cultivar. In addition, according to the existing research, exogenous SA could be applied as a potential growth regulator to improve grapevine water stress tolerance. Application of SA with 2 mM concentration would increase antioxidant enzymes activities and therefore reduce the detrimental effects of drought stress.

## Acknowledgements

We would like to thank and reserve in memory late Professor Rasoul Jalili Marandi.

## References

1. Abbate P.E, Dardanellib J.L, Cantarero M, Maturanoc M, Melchiorid R.J.M, Sueroa E.E. 2004. Climatic and water availability effects on water-use efficiency in wheat. *Journal of Crop Science* 44, 474-483.
2. Abdulah A, Ghamdi A. 2009. Evaluation of oxidative stress in two wheat (*Triticum aestivum* L.) cultivars in response to drought. *Int. Journal of Agricultural and Biological Engineering* 11, 7-12.
3. Aebi H. 1984. Catalase in vitro. *Method Enzymol* 105, 121-126.
4. Agamy R.A, Hafez E.E, Taha T.H. 2013. Acquired resistant motivated by SA applications on salt stressed tomato (*Lycopersicon esculentum* Mill). *American- Eurasian Journal of Agricultural & Environmental Sciences* 13(1), 50-57.
5. Al-Hakimi A.M.A, Hamada A.M. 2001. Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamin or sodium salicylate. *Journal of Plant Biology* 44, 253-261.
6. Arora A, Singh V.P, Mohan J. 2001. Effect of nitrogen and water stress on photosynthesis and

- nitrogen content in wheat. *Biologia Plantarum* 44(1), 153-155
7. Asada K. 1992. Ascorbate peroxidase and hydrogen peroxide scavenging enzyme in plants. *Journal of Plant Physiology* 30, 987-998.
  8. Asada K, Takahashi M. 1987. Production and scavenging of active oxygen in photosynthesis. In: Kyle DJ Osmond CB Arntzen CJ (eds.) *Photo-inhibition topics in photosynthesis*. Elsevier Amsterdam 9, 227-287.
  9. Ashraf M.Y, Mazhar H, Naqvi L, Khan A.H. 1996. Effect of water stress on total phenols, peroxidase activity, Growth and yield on tomato. *Acta Horticulturae* 516, 41-45.
  10. Azooz M.M, Ismail A.M, Elhamd M.F. 2009. Growth, lipid peroxidation and antioxidant enzyme activities as a selection criterion for salt tolerance of maize cultivars grown under salinity stress. *International Journal of Agricultural and Biological Engineering* 11, 21-26.
  11. Bian S, Jiang Y. 2009. Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of Kentucky bluegrass in response to drought stress and recovery. *Scientia Horticulturae* 120, 264-270.
  12. Bradford M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Annual Review of Biochemistry* 72, 248-254.
  13. Chae H.S, Faureand F, Kieber J.J. 2003. The *eto1*, *eto 2*, and *eto 3* mutations and cytokinin treatment increase ethylene biosynthesis in *Arabidopsis* by increasing the stability of ACS Protein. *The Plant Cell* 15, 545-559.
  14. Chen Z, Iyer S, Caplan A, Klessig D.F, Fan B. 1997. Differential accumulation of SA and salicylic acid sensitive catalase in different rice tissue. *Journal of Plant Physiology* 114, 193-201.
  15. Cunhuva S, Wei D, Xiangling C, Xinna X, Yahong Z, Dong S, Jianjie S. 2010. The Effects of drought stress on the activity of acid phosphatase and its protective enzymes in pigweed leaves. *African Journal of Biotechnology* 9, 825-833.
  16. Cornelia P, Petrus A, Pop L, Chis A, Bandici G.E. 2010. Exogenous SA involvement on some physiological parameters amelioration in salt stressed wheat (*Triticum aestivum*) plantlets. *Analele Universitatii din Oradea, Fascicula: Protectia Mediului* 15, 160- 165.
  17. Cramer G.R, Lauchli A, Epstein E. 1986. Effect of NaCl and CaCl<sub>2</sub> on ion activities in complex nutrient solution and root growth of cotton. *Journal of Plant Physiology* 81, 792-797.
  18. Decov I, Tsonev T, Yordanov I. 2000. Effects of water stress and high-temperature stress on the structure and activity of photosynthetic apparatus of *Zea mays* and *Helianthus annuus*. *Photosynthetica* 38, 361-366.
  19. Dellongo O.T, Gonzales G.A, Pastori G.M. 1993. Antioxidant defenses sensitivity to drought. *Plant and Cell Physiology* 34(7), 1023-1028.
  20. Doulatabadian A, Modarressanavy S.A.M, Etemadi F. 1386. Effect of pretreatment of SA on wheat (*Triticum aestivum* L.) seed germination under salt stress. *Iranian Journal of Basic Medical Sciences* 4, 15-21.
  21. Efeoglu B, Ekmekci Y, Çicek N. 2009. Physiological responses of three maize cultivars to drought stress and recovery. *South African Journal of Botany* 75(1), 34-42.
  22. Elizabeth M.A, Boach S. 2008. SA may be involved in the regulation of drought-induced leaf senescence in perennials. *Environmental and Experimental Botany* 64, 105-112.
  23. El\_Tayeb M.A. 2005. Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regulation* 45, 215-224.
  24. Fariduddin Q, Hayat S, Ahmad A. 2003. SA influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brassica juncea* L. *Photosynthetica* 41, 281-284.
  25. Farooq M, Wahid A, Kobayashi N, Fujita D, Basra S.M.A. 2009. Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development* 29, 185-212.
  26. Filella I, Llusia J, Pin J.O, Pen J.U. 1998. Leaf gas exchange and fluorescence of *Phillyrea latifolia*, *Pistacia lentiscus* and *Quercus ilex* saplings in severe drought and high temperature conditions. *Environmental and Experimental Botany* 39, 213-220.
  27. Gharib F.A. 2006. Effect of SA on the growth, metabolic activities and oil content of basil and marjoram. *International Journal of Agricultural and Biological Engineering* 4, 485-492.
  28. Gill S.S, Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* 48, 909-930.
  29. Hajheidari M, Abdollahian- Noghabi M, Askari H. 2005. Proteome analysis of sugar beet leaves under draught stress. *Proteomics* 5, 463-99.

30. Hasheminasab H, Assad M.T, Aliakbari A, Sahhafi R. 2012. Influence of drought stress on oxidative damage and antioxidant defense systems in tolerant and susceptible wheat genotypes. *Journal of Agricultural Science* 4(8), 20-30.
31. Hayat S, Ahmad A. 2007. Salicylic acid: A plant hormone. Springer Publ, Dordrecht.
32. Hayat S, Hasan S.A, Fariduddin Q, Ahmad A. 2008. Growth of tomato (*Lycopersicon sculentum* L.) in response to SA under water stress. *Journal of Plant Interactions* 3, 297-304.
33. Hayat Q, Hayat S, Irfan M, Ahmad A. 2010. Effect of exogenous SA under changing environment: A review. *Environmental and Experimental Botany* 68, 14-25.
34. Heath R.L, Packer L. 1968. Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. *Biochemistry and Biophysics* 125, 189-198.
35. Horvath E, Pal M, Szalai G. 2007. Exogenous 4-hydroxy benzoic acid and SA modulate the effect of short-term drought and freezing stress on wheat plants. *Biologia Plantarum* 51, 480-487
36. Horvath E, Szalai G, Janda T. 2007. Induction of abiotic stress tolerance by SA signaling. *Journal of Plant Growth Regulation* 26, 290-300.
37. Hussain M, Malik M.A, Farooq M, Ashraf M.Y, Cheema M.A. 2008. Improving Drought tolerance by exogenous application of glycine betaine and SA in sunflower. *Journal of Agronomy and Crop Science* 194, 193-199.
38. Janda T, Horvat E, Szalai G, Paldi E. 2007. Role of SA in the induction of abiotic stress tolerance. *Biologia Plantarum* 51, 471-477.
39. Josaph B, Jini D, Sujatha S. 2010. Insight into role of exogenous SA on plants growth under salt environment. *Asian Journal of Crop Science* 2(4), 226-235.
40. Juan M, Rivero R.M, Romero L, Ruiz J.M. 2005. Evaluation of some nutritional and biochemical indicators in selecting salt-resistant tomato cultivars. *Environmental and Experimental Botany* 54, 193-201.
41. Kadioglu A, Saruhan N, Saglam A, Terzi R, Acet T. 2011. Exogenous SA alleviates effects of long term drought stress and delays leaf rolling by inducing antioxidant system. *Plant Growth Regulation* 64, 27-37.
42. Kavar T, Maras M, Kidric M, Sustar-Vozlic J, Meglic V. 2007. Identification of genes involved in the response of leaves of *Phaseolus vulgaris* to drought stress. *Molecular Breeding* 21, 159-172.
43. Kaya M.D, Okçub G, Ataka M, Çkılıç Y, Kolsarıcıa O. 2006. Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *The European Journal of Agronomy* 24, 291-295.
44. Khan W, Balakrishnan P, Smith D.L. 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. *Journal of Plant Physiology* 160(5), 485-492.
45. Khan M.I.R, Syeed S, Nazarand R, Anjum N.A. 2012. An insight into the role of SA and jasmonic acid in salt stress tolerance. In: *Phytohormones and abiotic stress tolerance in plants*. Springer 277-300.
46. Khodary S.E.A. 2004. Effect of SA on growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. *International Journal of Agricultural and Biological Engineering* 6, 5-8.
47. King H.M, Saltveit M.E. 2002. Chilling tolerance of maize, cucumber and rice seedling leaves and roots are differentially affected by salicylic acid. *Journal of Plant Physiology* 115, 571-576.
48. Kumar P, Dube S.D, Chauhan V.S. 1999. Effect of SA on growth, development and some biochemical aspect of soybean (*Glycine max* L. Merrill). *International Journal of Plant Physiology and Biochemistry* 4, 327-330.
49. Lazaridou M, Kirilov A, Noitsakis B, Todorov N, Katerov I. 2003. The effect of water deficit on yield and water use efficiency of lucerne. Optimal forage systems for animal production and the environment, Proceedings of the 12th Symposium of the European Grassland Federation, Pleven, Bulgaria.
50. Lazaridou M, Koutroubas S.D. 2004. Drought effect on water use efficiency of berseem clover at various growth stages. New directions for a diverse planet: Proceedings of the 4th International Crop Science Congress Brisbane, Australia. 26 Sept–1 Oct 2004.
51. Lichtenthaler H.K, Wellburn A.R. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions* 11, 591-592.
52. Manivannan P, Abdul Jaleel C, Somasundaram R, Panneersel R. 2008. Osmoregulation and antioxidant metabolism in drought-stressed *Helianthus annuus* L. under triadimefon

- drenching. The Comptes rendus Biologies 331, 418-425.
53. Meirs S, Philisohadas S, Aharoni N. 1992. Ethylene increased accumulation of fluorescent lipid- per oxidation products detected during parsley by a newly developed method. Journal of the American Society for Mass Spectrometry 117, 128-132.
54. Mohammadi A, Habibi D, Rihami M, Mafakheri S. 2011. Effect of Drought Stress on Antioxidant Enzymes Activity of Some Chickpea Cultivars *American-Eurasian L. American-Eurasian journal of agricultural & environmental sciences* 11(6), 782-785.
55. Mohammadkhani N, Heidari R. 2008. Effects of drought stress on soluble proteins in two maize varieties. *The Turkish Journal of Biology* 32, 23-30.
56. Moharekar S.T, Lokhande S.D, Hara T, Tanaka R, Tanaka A, Chavan P.D. 2003. Effect of SA on chlorophyll and carotenoid contents of wheat and moongcaryopsis. *Photosynthetica* 41, 315-317.
57. Moussa H, Abdel-Aziz S.M. 2008. Comparative Response of Drought Tolerant and Drought Sensitive Maize Genotypes to Water Stress. *Australian Journal of Crop Science* 1, 31-36.
58. Nazar R, Iqbal N, Syeed S, Khan N.A. 2011. SA alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. *Journal of Plant Physiology* 168, 807-815.
59. Nguyen H.T, Babu R.C, Blum A. 1997. Breeding for drought resistance in rice: Physiology and molecular genetics considerations. *Crop Science* 37, 1426-1434.
60. Qin J, Wang X, Hu F, Li H. 2010. Growth and physiological performance responses to drought stress under non-flooded rice cultivation with straw mulching. *Plant, Soil and Environment* 56(2), 51-59.
61. Rubio M.C, Minchin F.R. 2002. Effect of water stress on antioxidant enzyme of leaves and nodules of transgenic alfalfa over-expressing superoxide dismutase. *Journal of Plant Physiology* 115, 531-540.
62. Sagee O, Riov J, Goren R. 1989. Ethylene-enhanced catabolism of (<sup>14</sup>C) indole-3- acetic acid to indole-3-carboxylic acid in citrus leaf tissues. *Journal of Plant Physiology* 191, 54-60.
63. Sairam R.K, Deshmukh P.S, Saxena D.C. 1998. Role of antioxidant systems in wheat genotype tolerance to water stress. *Biologia Plantarum* 41(3), 387-394b.
64. Sarkar S, Topha C. 1974. Effects of ethylene on the phenylalanine ammonia-lyase activity of carrot tissues. *Journal of Plant Physiology* 32(4), 318.
65. Sedghi M, Sharifi R.S, Pirzad A.R, Balaneji B.A. 2012. Phytohormonal regulation of antioxidant systems in petals of drought stressed pot marigold (*Calendula officinalis* L.). *Journal of Agricultural Science and Technology* 14, 869-878.
66. Senaratna T, Touchell D, Bunn T, Dixon K. 2000. Acetyl SA (Aspirin) and SA induce multiple stress tolerance in bean and tomato plants. *Plant Growth Regulation* 30, 157-161.
67. Shao H.B, Chu L.Y, Wu G, Zhang J.H, Lu Z.H, Hu Y.C. 2007. Changes of some anti-oxidative physiological indices under soil water deficits among 10 wheat (*Triticum aestivum* L.) genotypes at tillering stage. *Journal of Colloid and Interface Science* 54, 143-149.
68. Shakirova F.M, Sakhabutdinova A.R, Bezrukova M.V, Fathudinova R.A, Fathutdinova D.R. 2003. Insight into the role of exogenous salicylic acid on plants grown under salt environment. *Plant Science* 164, 317.
69. Sharma P, Dubey R.S. 2005. Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. *Plant Growth Regulation* 46, 209-221.
70. Sing B, Usha K. 2003. SA induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regulation* 39, 137-141.
71. Smart D.R, Carlise E, Goebel M, Nunez A. 2005. Transverse hydraulic redistribution by a grapevine. *Plant, Cell and Environment* 28, 157-166.
72. Smirnov N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. *The New Phytologist Trust* 125, 27-58.
73. Syeed S, Anjum N.A, Nazar R, Iqbal N, Masood A, Khan N.A. 2011. Salicylic acid-mediated changes in photosynthesis, nutrients content and antioxidant metabolism in two mustard (*Brassica juncea* L.) cultivars differing in salt tolerance. *Acta Physiologiae Plantarum* 33, 877-886.
74. Szepesi A, Csiszar J, Bajkan S, Gemes K, Horvath F, Erdel L, Deer A.K, Simon M.L, Tari L. 2005. Role of SA pre-treatment on the acclimation of tomato plants to salt and osmotic stress. *Acta Biologica Szegediensis* 49, 123- 125.

75. Uupdhyaya A, Sankhla D, Davis T.D, Sankhla N, Smidh B.N. 1985. Effect of paclobutrazol on the activities of some enzymes of activated oxygen metabolism and lipid peroxidation in senescing soybean leaves. *Journal of Plant Physiology* 121, 453-461.
76. Vallivodan B, Nguyen H.T. 2006. Understanding regulatory networks and engineering for enhanced drought tolerance in plants. *Current Opinion in Plant Biology* 9, 189-195.
77. Wang Y.S, Wang J, Yang Z.M, Wang Q.Y, Lu B, Li S.Q, Lu Y.P, Wang S.H, Sun X. 2004. SA modulates aluminum-induced oxidative stress in roots of *Cassia tora* L. *Acta Botanica Sinica* 46, 819-828.
78. Wang W.B, Kim Y.H, Lee H.S, Kim K.Y, Deng X.P, Skwak S. 2009. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stress. *Plant Physiology and Biochemistry* 47, 570-577.
79. Wise M.J, Tunnacliffe A. 2004. POPP the question: what do LEA proteins do? *Trends in Plant Sciences* 9, 7-13.
80. Zhou Zh.Sh, Guo K, Abdou Elbaz A, Yang Zh M. 2009. SA alleviates mercury toxicity by preventing oxidative stress in roots of *Medicago sativa*. *Environmental and Experimental Botany* 65, 27-34.