

ARTICLE

Exogenous salicylic acid alleviates oxidative damage of barley plants under drought stress

Ghader Habibi*

Department of Biology, Payame Noor University, Tehran, Iran

ABSTRACT This paper reports the effects of 500 μM salicylic acid (SA) application on drought stress acclimation of barley (*Hordeum vulgare* L. cv Nosrat) plants grown in soil culture. In these experiments the following treatments were used: CK (control), DR (drought), SA (500 μM) and DSA (SA+drought). The results showed that drought stress decreased the dry mass and net CO_2 assimilation rate (A) of plants, which were all increased by the addition of SA. Under drought conditions, the improvement of photosynthesis of barley plants treated with SA was associated with an increase in g_s , whereas the maximal quantum yield of PSII (F_v/F_m) did not change with SA treatment. Malondialdehyde (MDA) content remained unchanged in DSA plants because of an efficient scavenging of reactive oxygen species (ROS) following a significant enhancement of some antioxidative enzyme activities. The present work suggests that the improvement of SA on drought tolerance of barley plants was associated with the increase of antioxidant defense abilities and maintenance of photosynthesis under drought, which may elucidate the physiological mechanism of SA in improvement of drought tolerance of barley plants.

Acta Biol Szeged 56(1):57-63 (2012)**KEY WORDS**antioxidative enzymes
drought stress
Hordeum vulgare L.
net CO_2 assimilation rate (A)
salicylic acid

Stress factors such as drought trigger common reactions in plants and lead to cellular damages mediated by reactive oxygen species (ROS) (Mano 2002). Oxidative stress results in a serious imbalance between the production and removal of ROS. Accumulation of ROS induces oxidative damage to proteins, membrane lipids and other cellular components (Creissen and Mullineaux 2002). It also inhibits the photochemical activities and decreases the activities of enzymes in the Calvin cycle (Monakhova and Chernyad'ev 2002).

Under environmental stress conditions such as drought, high activities of antioxidant enzymes and high levels of nonenzymatic antioxidant compounds are important for plants to tolerate stresses (Gong et al. 2005). However, responses of antioxidative enzymes to water deficiency is variable and depends on the intensity of the imposed water stress (Habibi and Hajiboland 2011). Antioxidative enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) play an important role against oxidative stress (Apel and Hirt 2004). Plants containing high activities of antioxidant enzymes have shown considerable resistance to oxidative damage caused by ROS (Khan et al. 2007; Gapinska et al. 2008; Frary et al. 2010).

Salicylic acid (SA) is a naturally occurring plant hormone, influences various physiological and biochemical functions in plants. It can act as an important signaling molecule and

has diverse effects on tolerance to biotic and abiotic stresses (Arfan et al. 2007; Wang et al. 2010). Its role in plant tolerance to abiotic stresses such as ozone, heat, heavy metal and osmotic stress (El-Tayeb 2005; Szepesi et al. 2005; Wang et al. 2010; Liu et al. 2011; Kadioglu et al. 2011) has been reported by several authors.

A survey of literature indicates that salicylic acid plays a key role in providing tolerance to the plants, exposed to water stress (Hayat et al. 2008; Kadioglu et al. 2011). Salicylic acid was found to enhance the activities of antioxidant enzymes such as peroxidase (POD), SOD and CAT, when sprayed exogenously to the drought stressed plants of tomato (Hayat et al. 2008) or to the salinity stressed plants (Szepesi et al. 2008; Yusuf et al. 2008). The exogenous SA application also enhanced the growth and photosynthetic rate in wheat (Hussein et al. 2007) under water stress. However, numerous studies have demonstrated that the effect of exogenous SA depends on various factors, including the species and developmental stage, the mode of application and the concentration of SA (Vanacker et al. 2001; Horváth et al. 2007).

In recent years the role of SA in the induction of tolerance against paraquat-induced oxidative damage in barley has been described (Ananieva et al. 2004). With respect to drought stress, relevant work is limited especially in case of barley, which is an important crop plant with a number of agro-industrial uses. This is of great interest since increasing the amount of irrigated land is difficult; water is scarce and only the most efficient agricultural systems are likely to receive

Accepted May 11, 2012

*Corresponding author. E-mail: gader.habibi@gmail.com

inputs of irrigation water (Feres et al. 2003). There is no information about the physiological responses of the barley (*cv* Nosrat) to SA under water stress, which is supposed to influence many physiological processes in drought stressed plants. The aim of this study was to investigate the effect of salicylic acid on barley growth, photosynthesis and defense system under drought stress. In addition of monitoring the growth, relative water content, chlorophyll fluorescence parameters and gas exchange pattern, the present work examined the effect of SA on the antioxidant defense system during drought stress in barley plants.

Materials and Methods

Plant material and treatments

Barley (*Hordeum vulgare* L. *cv* Nosrat) seeds were planted in pots (14 cm in diameter and 105 cm in depth), each pot containing 15 kg of sandy loam soil. The field capacity (FC) of soil was measured by gravimetric method. Before sowing, 15 kg of soil was fertilized, and 200 mg nitrogen kg⁻¹ soil as NH₄NO₃ and 50 and 62.5 mg phosphorus and potassium kg⁻¹ soil as KH₂PO₄ were applied to the soil cultures. The following treatments were used in the experiments: CK (control), DR (drought), SA (500 μM) and DSA (SA+drought). Water stress was initiated by withholding irrigation. Control plants were grown at 90% and drought stressed plants at 40% of FC. Plants grown in pots were kept in a greenhouse under natural day/night conditions with photosynthetically active radiation (PAR) of 800 ± 100 μmol m⁻² s⁻¹ and average day/night temperature of 30 ± 2/18 ± 2°C. After emergence, the seedlings were thinned to forty plants per pot and watered every 4-5 days to maintain 90% FC of the soil until fifteen days after sowing. From then on, the seedlings were watered every 1-2 days to maintain 90% FC in the CK soil and 40% FC in the drought treatments (DR and DSA). Salicylic acid (SA) was dissolved in absolute ethanol then added drop wise to water (ethanol/water: 1/1000 v/v) (Williams et al. 2003). Fifteen days after sowing, SA was applied on the foliage at a concentration of 500 μM with a hand sprayer. The appropriate controls without SA treatment were sprayed with ethanol/water (1/1000 v/v). The volume of the spray was 25 ml per pot. At 30 days after sowing (15 d after SA application and drought treatment), measurements were done and the young fully expanded leaves were collected and frozen in liquid N₂ immediately until analysis.

Analysis of water relations

Leaves were washed with distilled water, blotted dry on filter paper and after determination of fresh weight (FW) were dried for 48 h at 70°C for determination of dry weight (DW). Relative water content (RWC) was measured and calculated according to Lara et al. (2003) all in the second youngest leaf harvested at 1 h after light on in the greenhouse.

Measurements of photosynthetic gas exchange and chlorophyll fluorescence

Before harvest gas exchange parameters were measured. Net CO₂ fixation (A , μmol m⁻² s⁻¹), transpiration rate (E , mmol m⁻² s⁻¹) and stomatal conductance to water vapor (g_s , mol m⁻² s⁻¹) were measured with a calibrated portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK) after 5 h of the light period and sealed in the leaf chamber under a photon flux density of 800 ± 100 μmol m⁻² s⁻¹ in greenhouse conditions. Chlorophyll fluorescence parameters were recorded using a portable fluorometer (OSF1, ADC Bioscientific Ltd., UK) for both dark adapted and light adapted leaves. Leaves were acclimated to dark for 30 min using leaf clips before measurements were taken. Initial (F_0), maximum (F_m), variable ($F_v = F_m - F_0$) fluorescence as well as maximum quantum yield of PSII (F_v/F_m) were recorded. Light adapted leaves (400 μmol m⁻² s⁻¹) were used for measurement of "steady-state" (F_s) and maximum (F'_m) fluorescence. Calculations were made for F'_0 ($F'_0 = F_0 / [(F_v/F_m) + (F_0/F'_m)]$), effective quantum yield of PSII, $\Phi_{PSII} [(F'_m - F_s)/F'_m]$, photochemical quenching, $qP [(F'_m - F_s)/(F'_m - F'_0)]$ and non-photochemical quenching, $qN (1 - [(F'_m - F'_0)/(F'_m - F_0)])$ (Krall and Edwards 1992).

Assay of enzymes activity and related metabolites

Determination of the activity of antioxidant enzymes and concentration of related metabolites were undertaken according to the methods described by Habibi and Hajiboland (2010). Fresh samples were ground in the presence of liquid nitrogen and measurements were undertaken using spectrophotometer (Specord 200, Analytical Jena, Germany). Superoxide dismutase (SOD, EC 1.15.1.1) activity was estimated according to the method of Giannopolitis and Ries (1977). Enzyme was extracted in 25 mM HEPES pH 7.8 with 0.1 mM EDTA and the supernatant was added to the reaction mixture containing 0.1 mM EDTA, 50 mM Na₂CO₃ (pH 10.2), 13 mM methionine, 63 μM nitroblue tetrazolium chloride (NBT), 13 μM riboflavin. One unit of SOD was defined as the amount of enzyme which produced a 50% inhibition of NBT reduction under assay conditions. For the determination of catalase (CAT, EC 1.11.1.6) activity, samples were homogenized with 50 mM phosphate buffer pH 7.0 and assayed spectrophotometrically by following the degradation of H₂O₂ at 240 nm according to the method of Simon et al. (1974). Reaction medium contained 50 mM phosphate buffer (pH 7) and 10 mM H₂O₂. Peroxidase (POD, EC 1.11.1.7) activity was determined using the guaiacol test at 470 nm (Chance and Maehly 1995). The enzyme was extracted by 10 mM phosphate buffer pH 7.0 and assayed in a solution containing 10 mM phosphate buffer, 5 mM H₂O₂ and 4 mM guaiacol. Ascorbate peroxidase (APX, EC 1.11.1.11) activity was assayed by following reduction in absorbance at 290 nm as ascorbate was oxidized according to the method of Boominathan and Doran (2002). The reaction

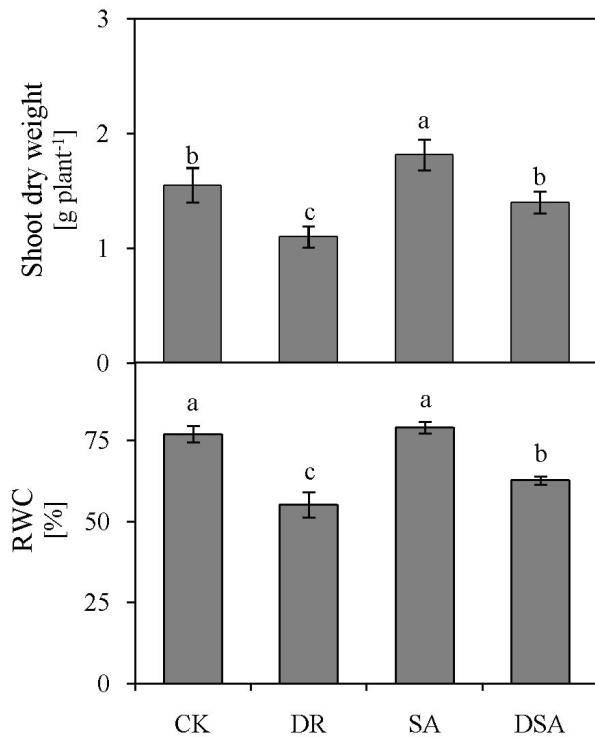


Figure 1. Dry weight (mg plant⁻¹) of leaves and relative leaf water content (RWC, %) under different treatments: CK (control), DR (drought), SA (500 μM) and DSA (SA+drought). Each value is the mean ± SD of 4 replicates. Data of each column indicated by the same letters are not significantly different ($P < 0.05$).

mixture contained 50 mM phosphate buffer (pH 7), 0.2 mM EDTA, 0.5 mM ascorbic acid and 50 μg bovine serum albumin (BSA). Lipid peroxidation was estimated at 532 nm from the amount of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid (Sigma). MDA levels were calculated from a 1,1,3,3-tetraethoxypropane (Sigma) standard curve. The concentration of H₂O₂ was determined using potassium titanium-oxalate at 508 nm (Habibi et al.

2010). Soluble protein was estimated spectrophotometrically by the Bradford method (1976).

Statistical analysis

Experiments were undertaken in complete randomized block design with 4 replications. Statistical analysis was carried out using Sigma Stat (3.5) with Tukey test ($P < 0.05$). Correlation analysis using Spearman Rank Order Correlation in Sigma Stat (3.5) were conducted to determine the relationship between maximum quantum yield of PSII (F_v/F_m) and stomatal conductance (g_s).

Results

Both relative water content (RWC) and dry weight decreased dramatically in water-stressed plants (Fig. 1). In contrast to drought, SA spraying increased dry matter accumulation in well-watered plants, as compared with control plants. Moreover, the plants after SA treatment showed higher dry weight and water content compared to plants without application of SA under drought conditions. Salicylic acid treatment did not increase relative water content in well-watered plants, as compared with controls.

The study of PSII photochemistry in the dark-adapted leaves showed that there was no significant difference in the maximal quantum yield of PSII (F_v/F_m) between control and SA-treated plants under well-watered conditions (Table 1). However, reduction of maximal efficiency of PSII in dark-adapted leaves (F_v/F_m) and effective quantum yield of PSII (Φ_{PSII}) were detectable in leaves of water-stressed plants. In addition, there was a good linear correlation ($r = 0.84$, $P < 0.01$) between stomatal conductance to water vapor (g_s) and F_v/F_m in water-stressed plants (Fig. 4). Photochemical quenching (qP) and non-photochemical quenching (qN) were not influenced under SA spraying and drought conditions. However, a decrease in qN was observed in SA applied plants compared with those without application of SA under drought conditions.

Table 1. Leaf physiological traits of barley plants under different treatments: CK (control), DR (drought), SA (500 μM) and DSA (SA+drought). A net photosynthetic rate, E transpiration rate, g_s stomatal conductance, WUE (A/E) water use efficiency, F_v/F_m maximum quantum yield of PSII, qP photochemical quenching, qN non-photochemical quenching, Φ_{PSII} effective quantum yield of PSII. Each value is the mean ± SD of 4 replicates. Data of each row indicated by the same letters are not significantly different ($P < 0.05$).

Photochemistry	CK	DR	SA	DSA
F_v/F_m	0.83 ± 0.01 ^a	0.77 ± 0.04 ^b	0.84 ± 0.01 ^a	0.80 ± 0.02 ^{ab}
qP	0.96 ± 0.01 ^a	0.94 ± 0.06 ^a	0.97 ± 0.02 ^a	0.96 ± 0.03 ^a
qN	0.21 ± 0.03 ^{ab}	0.31 ± 0.08 ^a	0.19 ± 0.08 ^b	0.22 ± 0.05 ^a
Φ_{PSII}	0.78 ± 0.02 ^{ab}	0.74 ± 0.03 ^b	0.81 ± 0.02 ^a	0.74 ± 0.05 ^b
Gas exchange	CK	DR	SA	DSA
A (μmol m ⁻² s ⁻¹)	12.6 ± 2.13 ^{ab}	4.83 ± 1.54 ^c	15.8 ± 1.49 ^a	9.53 ± 1.92 ^b
E (mmol m ⁻² s ⁻¹)	4.81 ± 0.38 ^{ab}	3.54 ± 1.10 ^b	5.53 ± 0.45 ^a	4.99 ± 0.61 ^{ab}
g_s (mol m ⁻² s ⁻¹)	0.41 ± 0.02 ^b	0.26 ± 0.01 ^c	0.49 ± 0.12 ^a	0.41 ± 0.03 ^b
WUE (μmol mmol ⁻¹)	2.61 ± 0.34 ^{ab}	1.37 ± 0.16 ^c	2.86 ± 0.20 ^a	1.95 ± 0.59 ^{bc}

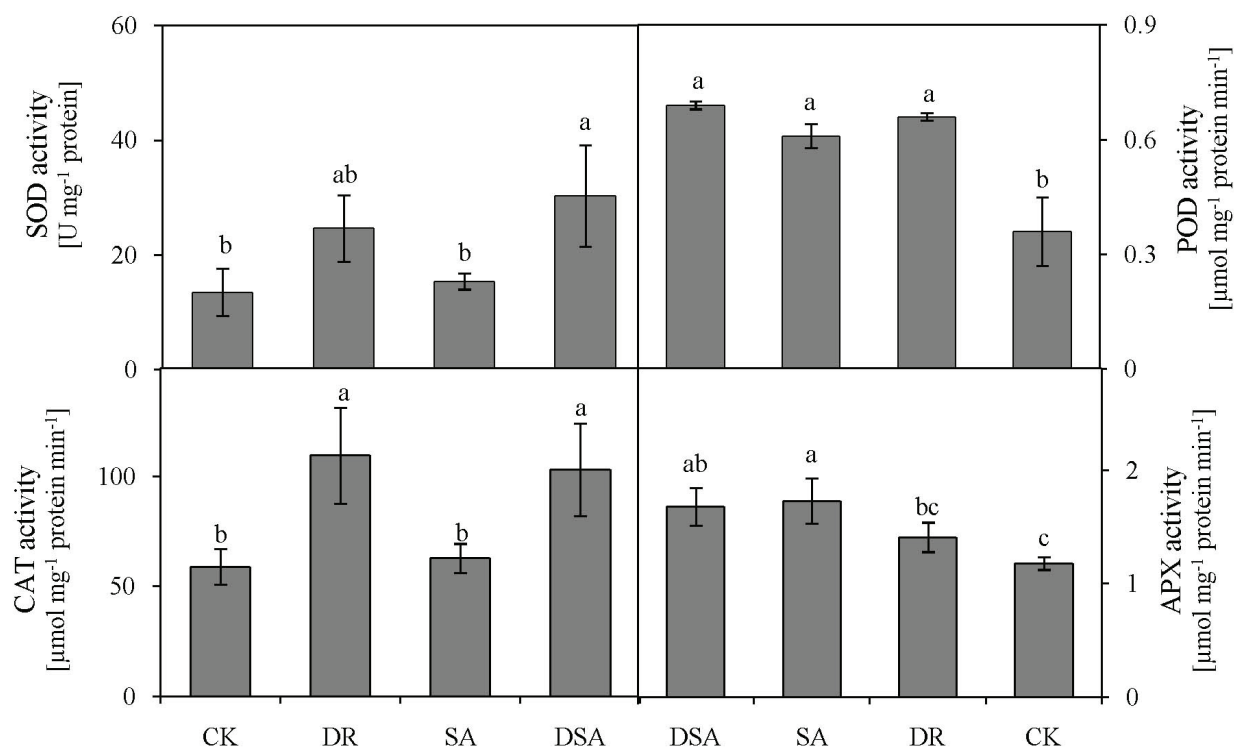


Figure 2. Antioxidant index of barley plants under different treatments: CK (control), DR (drought), SA (500 μM) and DSA (SA+drought). SOD superoxide dismutase, CAT catalase, POD peroxidase, APX ascorbate peroxidase. Each value is the mean ± SD of 4 replicates. Bars indicated with the same letter are not significantly different ($P < 0.05$).

Net CO₂ assimilation rate (A) was not influenced by SA spraying, but was reduced by drought (Table 1). In this study, a remarkable reduction in leaf dry weight in drought stressed plants was associated with significant reduction of A . In addition, a significantly rise in the net assimilation rate was observed in the SA-supplemented water-stressed samples relative to water-deficit treatment. Transpiration rate (E) was only slightly affected by water stress, and this slight reduction was alleviated by SA under water stress. Stomatal conductance (g_s) was reduced strongly under drought conditions but increased by SA. SA-treated plants still maintain higher g_s value compared to those without application of SA under drought, which indicated that application of SA had positive effects on stomatal conductance of barley plants. Water use efficiency was significantly lower in drought-stressed plants. Thus, compared with the transpiration rate, the water use efficiency showed a greater decrease during water deficit.

Drought stress caused a significant increase of POD and CAT activities relative to control plants, whereas SOD and APX activities did not change (Fig. 2). Under well-watered conditions, SA stimulated APX and POD activities in comparison with the control, whereas CAT and SOD activities were not affected. Activity of APX and POD in water-stressed plants did not differ from that in SA-supplemented water-

deficit plants. However, a significantly rise in the activity of SOD and CAT was observed in the SA-supplemented water-deficit samples relative to water-deficit treatment. Drought stress without SA spraying caused significant accumulation of malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) (Fig. 3). Drought stress enhanced H₂O₂ content in both SA treated and non-SA treated plants under water stress conditions. In contrast to DR plants, in DSA plants, MDA level did not change at the end of the experiment.

Discussion

In this work, similarly with that observed for wheat (Hayat et al. 2005; Shakirova 2007) and maize (Khodary 2004), SA increased shoot dry weight in barley plants. Drought stress reduced growth activity of barley (Fig. 1), as it was observed in other plants species (Degu et al. 2008; Gao et al. 2011). It is a well-known fact that SA potentially generates a wide array of metabolic responses in plants and also affects plant water relations (Hayat et al. 2010). In this study, similarly with that observed for wheat (Singh and Usha 2003) and *Ctenanthe setosa* plants grown under drought conditions (Kadioglu et al. 2011), the results showed that barley plants sprayed with 500 μmol SA solution could maintain higher RWC compared with those plants which had not been treated with SA before

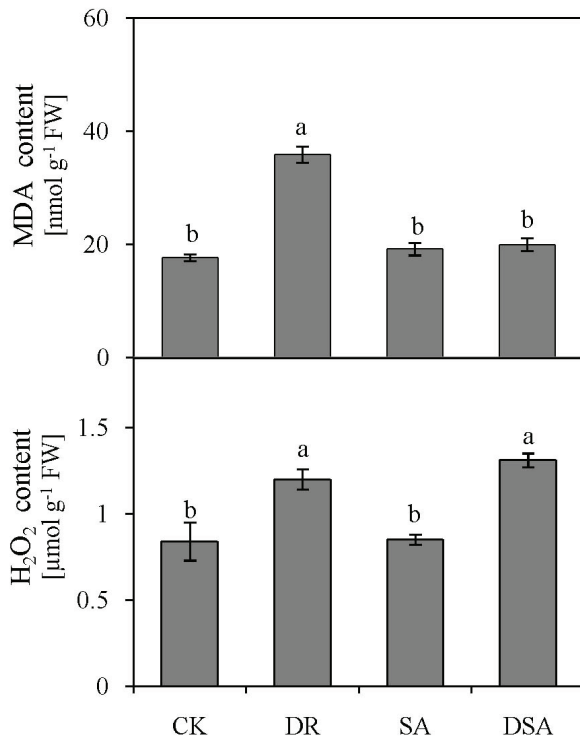


Figure 3. Leaf content of MDA (nmol g⁻¹ FW) and H₂O₂ (μmol g⁻¹ FW) in barley leaves at different treatments: CK (control), DR (drought), SA (500 μM) and DSA (SA+drought). Bars indicated with the same letter are not significantly different ($P < 0.05$).

drought stress. These results show that application of SA is useful for drought tolerance improvement of barley plants.

Many authors suggested that application of chlorophyll a fluorescence analysis as a reliable method to assess the changes in the function of PSII under stress conditions (Price and Hendry 1991; Broetto et al. 2007). Lower photosynthetic activity could be a consequence of low photochemical efficiency of PSII, as shown by its lower quantum yield (Pieters and Souki 2005). We found that the significant decrease in F_v/F_m was observed under water stress conditions, which was possibly due to the reduction of g_s and restriction of CO₂ for photosynthesis and indicated photoinhibition. These results are consistent with the findings of Ranjbarfordoei et al. (2006), Gunes et al. (2007) and Boughalleb and Hajlaoui (2011) that declining F_v/F_m values implies that photochemical conversion efficiency could indicate the possibility of photoinhibition.

Inhibition of photosynthesis of plants caused by drought stress includes stomatal and non-stomatal factors. However, which of them is the main factor is associated with plant species and stress conditions (Gong et al. 2005). In the present study, the stomatal density decreased significantly with water stress. Reduction of stomatal conductance (g_s) inhibits supply of CO₂ and consequently reduces CO₂ assimilation (A) is a

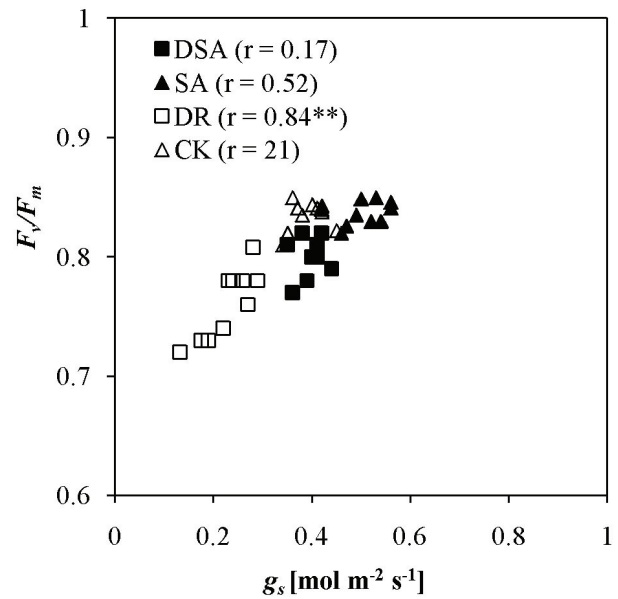


Figure 4. Correlation between maximum quantum yield of PSII (F_v/F_m) and stomatal conductance (g_s) in barley plants at different treatments. ns, *, and **: non-significant and significant, at the 5% and 1% levels of probability, respectively.

well known phenomenon in drought stressed plants (Lawlor and Cornic 2002). Reductions in photosynthetic performance under water stress have also been observed by Tognetti et al. (2005), Bacelar et al. (2006) and Ben Ahmed et al. (2009). The significant correlation between F_v/F_m and g_s confirmed the idea that stomatal closure that limits CO₂ availability for dark reactions may be one of mechanisms for photoinhibition in drought-stressed leaves. On the other hand, reduction of photosynthetic rate in drought-stressed plants in this work could not only be attributed to the stomatal limitation but may also be explained by inhibited leaf photochemistry as well as metabolic impairment *i.e.* reduction of Rubisco activity and ATP synthesis (Reddy et al. 2004).

Following the drought stress, SA-supplemented plants showed a higher g_s value in response to drought stress when compared with water-deficit plants. This is in accordance with the effect of SA on photosynthetic activity and stomatal conductance of tomato under salt stress (Poór et al. 2011). The present study shows that stomatal limitation is involved in SA-induced alleviation of the negative effects drought stress on photosynthesis in barley leaves. This conclusion was based on the observations that (1) SA treatment led to a significant increase in A in water-stressed plants, which was highly associated with the increase in g_s , and (2) F_v/F_m and effective quantum yield of PSII (Φ_{PSII}) did not change with SA treatment (Table 1). Therefore, under drought stress, the improvement of photosynthesis of barley plants exposed to SA was associated with stomatal factors.

The water status of the barley leaves was expressed by water use efficiency (WUE) and relative water content (RWC) parameters. WUE decreased significantly because of relatively high transpiration rate (E) under water stress in both treatments tested. The net photosynthetic rate and stomatal conductance of higher plants leaves are known to decrease as RWC decrease (Lawlor and Cornic 2002).

Plants are able to protect their tissues from the harmful effects of drought-accumulated reactive oxygen species (ROS) using enzymes such as SOD, APX and CAT (Verhagen et al. 2004). The results showed that SA has induced all antioxidant enzyme activities in DSA plants under drought condition, which may be related to the induction of antioxidant responses that protect the plant from oxidative damage. There is data supporting that SA increases the activity of antioxidant enzymes such as CAT, POD and SOD (Hayat et al. 2008 and 2010), which in turn protect plants against ROS generation and lipid peroxidation. On the other hand, it was reported an increase in activities of SOD and APX but an inhibition of CAT activity following SA treatment (Janda et al. 2003; Shakirova 2007). In this work, CAT activity did not decrease in SA-treated plants under well-watered condition. This finding was in agreement with those of Farooq et al. (2009), who reported that CAT inhibition by SA cannot be validated in all plants. In this work, a significant rise in the activity of CAT and SOD in the DSA samples relative to DR treatment revealed that SA exerts beneficial effects on stress tolerance of barely by enhancing their antioxidative capacity. The results showed that drought stress independently and in combination with SA led to an increase in the activity CAT and H_2O_2 content. The observed higher activity of the photorespiratory enzymes such as CAT in treated plants was most probably due to enhanced energy dissipation through photorespiration in the stressed plants (Yordanova and Popova 2007). These results are consistent with the findings of Agarwal et al. (2005) and Mora-Herrera et al. (2005) who revealed that H_2O_2 accumulation induced by SA was not related to inhibition of CAT activity. Amounts of MDA remained unchanged in SA-supplemented plants exposed to water-deficit because of an efficient scavenging of ROS following significant enhancement of SOD, CAT, POD and APX activities. It indicates that antioxidant defense system induced by SA may protect plants under water-stress conditions, while under drought without SA spraying, an imbalance between production and scavenging of ROS may cause oxidative stress as could be judged by the accumulation of MDA.

In summary, the results showed that drought stress decreased the plant dry mass and net CO_2 assimilation rate, which were all increased by addition of SA. This was associated with the increase of water content and enhancement of antioxidant enzymes activities of plants by addition of SA. The results of the present work also suggested that the improvement of SA on drought tolerance of barely plants was

associated with the increase of antioxidant defense capacity of tissues, alleviation of oxidative damage of functional molecules and maintenance of many physiological processes such as photosynthesis under drought. From this conclusion we can summarize that SA application can improve antioxidant defense system under drought stress conditions, and it may be recommended in arid and semiarid regions.

References

- Agarwal S, Sairam RK, Srivastava GC, Tyagi A, Meena RC (2005) Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings. *Plant Sci* 169:559-570.
- Arfan M, Athar HR, Ashraf M (2007) Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? *J Plant Physiol* 164:685-94.
- Ananieva EA, Christov KN, Popova LP (2004) Exogenous treatment with salicylic acid leads to increased antioxidant capacity in leaves of barley plants exposed to paraquat. *J Plant Physiol* 161:319-328.
- Apel K, Hirt H (2004) Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol* 55:373-399.
- Bacelar EA, Santos DL, Moutinho-Pereira JM, Gonçaves BC, Ferreira HF, Correia CM (2006) Immediate responses and adaptive strategies of three olive cultivars under contrasting water availability regimes: changes on structure and chemical composition of foliage and oxidative damage. *Plant Sci* 170:596-605.
- Ben Ahmed C, Ben Rouinab B, Sensoyc S, Boukhris M, Ben Abdallah F (2009) Changes in gas exchange, proline accumulation and antioxidative enzyme activities in three olive cultivars under contrasting water availability regimes. *Environ Exp Bot* 67:345-352.
- Boominathan R, Doran PM (2002) Ni induced oxidative stress in roots of the Ni hyperaccumulator, *Alyssum bertoloni*. *New Phytol* 156:202-205.
- Boughalleb F, Hajlaoui H (2011) Physiological and anatomical changes induced by drought in two olive cultivars (*cv* Zalmati and Chemlali). *Acta Physiol Plant* 33:53-65.
- Bradford MM (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248-254.
- Broetto F, Duarte HM, Lüttge Q (2007) Responses of chlorophyll fluorescence parameters of the facultative halophyte and C_3 -CAM intermediate species *Mesembryanthemum crystallinum* to salinity and high irradiance stress. *J Plant Physiol* 164:904-912.
- Degu HD, Ohta M, Fujimura T (2008) Drought tolerance of *Eragrostis tef* and development of roots. *Int J Plant Sci* 169:768-775.
- Chance B, Maehly AC (1995) Assays of catalases and peroxidases. *Methods Enzymol* 2:764-775.
- Creissen GP, Mullineaux PM (2002) The molecular biology of the ascorbate-glutathione cycle in higher plants. In Inzé D, Montanu MV, ed., *Oxidative stress in plants*. Taylor & Francis, UK, pp. 247-270.
- El-Tayeb MA (2005) Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regul* 45:215-224.
- Farooq M, Basra SMA, Wahid A, Ahmad N, Saleem BA (2009) Improving the drought tolerance in rice (*Oryza sativa* L.) by exogenous application of salicylic acid. *J Agron Crop Sci* 195:237-246.
- Fereres E, Goldhamer DA, Parsons LR (2003) Irrigation water management of horticultural crops. *Hort Science* 38:1036-1042.
- Frary A, Gol D, Keles D, Okmen B, Pinar H, Sigva HO, et al. (2010) Salt tolerance in *Solanum pennellii*: antioxidant response and related QTL. *BMC Plant Biol* 10:58.
- Fu J, Huang B (2001) Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environ Exp Bot* 45:105-114.
- Gapinska M, Sklodowska M, Gabara B (2008) Effect of short and long-term salinity on the activities of antioxidative enzymes and lipid peroxidation

- in tomato roots. *Acta Physiol Plant* 30:11-18.
- Gao D, Cai K, Chen J, Luo S, Zeng R, Yang J, Zhu X (2011) Silicon enhances photochemical efficiency and adjusts mineral nutrient absorption in *Magnaporthe oryzae* infected rice plants. *Acta Physiol Plant* 33:675-682.
- Gong H, Zhu X, Chen K, Wang S, Zhang C (2005) Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Sci* 169:313-321.
- Giannopolitis CN, Ries SK (1977) Superoxide dismutase. I. occurrence in higher plants. *Plant Physiol* 59:309-314.
- Gunes A, Inal A, Bagci EG, Coban S (2007) Silicon mediated changes on some physiological and enzymatic parameters symptomatic of oxidative stress in barley grown in sodic-B toxic soil. *J Plant Physiol* 164:807-811.
- Habibi G, Hajiboland R (2011) Comparison of water stress and UV radiation effects on the induction of CAM and antioxidative defense in the succulent *Rosularia elymaitica* (Crassulaceae). *Acta Biol Cracov Bot* 53:7-15.
- Habibi G, Hajiboland R, Dehghan G (2010) Contrastive response of *Phlomis tuberosa* to salinity and UV radiation stresses. *Acta Biol Szeged* 54:37-43.
- Habibi G, Hajiboland R (2010) Photosynthetic characteristics and antioxidative responses in three species of Crassulaceae following drought stress. *Journal of Sciences I.R. Iran* 21:205-212.
- Hayat Q, Hayat S, Irfan M, Ahmad A (2010) Effect of exogenous salicylic acid under changing environment: a review. *Environ Exp Bot* 68:14-25.
- Hayat S, Fariduddin Q, Ali B, Ahmad A (2005) Effect of salicylic acid on growth and enzyme activities of wheat seedlings. *Acta Agron Hung* 53:433-437.
- Hayat S, Hasan SA, Fariduddin Q, Ahmad A (2008) Growth of tomato (*Lycopersicon esculentum*) in response to salicylic acid under water stress. *J Plant Interact* 3:297-304.
- Horváth E, Szalai G, Janda T (2007) Induction of abiotic stress tolerance by salicylic acid signalling. *J Plant Growth Regul* 26:290-300.
- Hussein MM, Balbaa LK, Gaballah MS, (2007) Salicylic acid and salinity effects on growth of maize plants. *Res J Agric Biol Sci* 3:321-328.
- Janda T, Horvath G, Szalai G, Paldi E (2007) Role of salicylic acid in the induction of abiotic stress tolerance. In Hayat S, Ahmad A, eds., *Salicylic Acid, A plant Hormone*. Springer Publishers, Dordrecht, The Netherlands.
- Janda T, Szalai G, Rios-Gonzales K, Veisz O, Paldi E (2003) Comparative study of frost tolerance and antioxidant activity in cereals. *Plant Sci* 164:301-306.
- Kadioglu A, Saruhan N, Sağlam A, Terzi R, Acet T (2011) Exogenous salicylic acid alleviates effects of long term drought stress and delays leaf rolling by inducing antioxidant system. *Plant Growth Regul* 64:27-37.
- Khan NA, Samiullah, Singh S, Nazar R (2007) Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress. *J Agron Crop Sci* 193:435-444.
- Khodary SFA (2004) Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. *Int J Agric Biol* 6:5-8.
- Krall JP, Edwards GE (1992) Relationship between photosystem II activity and CO₂ fixation in leaves. *Physiol Plant* 86:180-187.
- Lara MV, Disante KB, Podesta FE, Andreo C, Drincovich MF (2003) Induction of a Crassulacean acid like metabolism in the C₄ succulent plant, *Portulaca oleracea* L.: physiological and morphological changes are accompanied by specific modifications in phosphoenolpyruvate carboxylase. *Photosynth Res* 77:241-254.
- Lawlor DW, Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ* 25:275-294.
- Li L, Staden JV (1998) Effect of plant growth regulators on the antioxidant system in callus of two maize cultivars subjected to water stress. *Plant Growth Regul* 24:55-66.
- Liu C, Guo J, Cui Y, Lü T, Zhang X, Shi G (2011) Effects of cadmium and salicylic acid on growth, spectral reflectance and photosynthesis of castor bean seedlings. *Plant Soil* 344:131-141.
- Mano J (2002) Early events in environmental stresses in plants induction mechanisms of oxidative stress. In Inzé D, Montganu MV, ed., *Oxidative stress in plants*. Taylor & Francis, UK, 217-246.
- Monakhova OF, Chernyadév II (2002) Protective role of kartinin-4 in wheat plants exposed to soil drought. *Appl Biochem Microbiol* 38:373-380.
- Mora-Herrera ME, Lopez-Delago H, Castillo-Morales A, Foyer CH (2005) Salicylic acid and H₂O₂ function by independent pathways in the induction of freezing tolerance in potato. *Physiol Plant* 125:430-440.
- Pieters AJ, El Souki S (2005) Effects of drought during grain filling on PSII activity in rice. *J Plant Physiol* 62:903-911.
- Poór P, Gémes K, Szepesi Á, Horváth F, Simon LM, Tari I (2011) Salicylic acid treatment via the rooting medium interferes with the stomatal response, CO₂ fixation rate and carbohydrate metabolism in tomato and decreases the harmful effects of subsequent salt stress. *Plant Biology* 13:105-114.
- Price AH, Hendry GAF (1991) Iron-catalysed oxygen radical formation and its possible contribution to drought damage in nine native grasses and three cereals. *Plant Cell Environ* 14:477-484.
- Ranjbarfordoei A, Samson R, Van Damme P (2006) Chlorophyll fluorescence performance of sweet almond [*Prunus dulcis* (Miller) D. Webb] in response to salinity stress induced by NaCl. *Photosynthetica* 44:513-522.
- Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought-induced responses of photosynthesis and metabolism in higher plants. *J Plant Physiol* 161:1189-1202.
- Shakirova FM (2007) Role of hormonal system in the manifestation of growth promoting and antistress action of salicylic acid. In Hayat S, Ahmad A ed., *Salicylic acid: a plant hormone*. Springer, Dordrecht, pp. 69-89.
- Singh B, Usha K (2003) Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regul* 39:137-141.
- Simon LM, Fatrai Z, Jonas DE, Matkovic B (1974) Study of peroxide metabolism enzymes during the development of *Phaseolus vulgaris*. *Biochem Physiol Pflanzen (BPP)* 166:387-392.
- Szepesi Á, Csiszár J, Bajkán Sz, Gémes K, Horváth F, Erdei L, Deér A, Simon LM, Tari I (2005) Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt- and osmotic stress. *Acta Biol Szeged* 49:123-125.
- Szepesi Á, Poór P, Gémes K, Horváth E, Tari I (2008) Influence of exogenous salicylic acid on antioxidant enzyme activities in the roots of salt stressed tomato plants. *Acta Biol Szeged* 52:199-200.
- Tognetti R, d'Andria R, Morelli G, Alvino A (2005) The effect of deficit irrigation on seasonal variations of plant water use in *Olea europaea* L. *Plant Soil* 273:139-155.
- Vanacker H, Lu H, Rate DN, Greenberg JT (2001) A role for salicylic acid and NPR1 in regulating cell growth in Arabidopsis. *Plant J* 28:209-216.
- Verhagen J, Put M, Zaai F, van Keulen H (2004) Climate change and drought risks for agriculture. *Environ Poll* 39:49-59.
- Wang LJ, Fan L, Loescher W, Duan W, Liu GJ, Cheng JS (2010) Salicylic acid alleviates decreases in photosynthesis under heat stress and accelerates recovery in grapevine leaves. *BMC Plant Biol* 10:34-40.
- Williams M, Senaratna T, Dixon K, Sivasithamparam K (2003) Benzoic acid induces tolerance to biotic stress caused by *Phytophthora cinnamomi* in *Banksia attenuata*. *Plant Growth Regul* 41:89-91.
- Yordanova R, Popova L (2007) Effect exogenous treatment with salicylic acid on photosynthetic activity and antioxidant capacity of chilled wheat plants. *Gen Appl Plant Physiol* 33:155-170.
- Yusuf M, Hasan SA, Ali B, Hayat S, Fariduddin Q, Ahmad A (2008) Effect of salicylic acid on salinity induced changes in *Brassica juncea*. *J Integrative Plant Biol* 50:1-4.