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# Effect of drought stress and selenium spraying on photosynthesis and antioxidant activity of spring barley

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**ABSTRACT**

This paper reports the effects of selenium (Se) application on some physiological characteristics of barley (*Hordeum vulgare* L. cv. Rihane-03) exposed to drought stress. Foliar application to barley at 30 g selenium $ha^{-1}$, as sodium selenate, increased significantly shoot dry weight and relative water content in well-watered plants. A remarkable reduction in dry weight of water-stressed plants was associated with significant decrease in maximal efficiency of PSII ($F_v/F_m$), stomatal conductance ($g_s$) and net $CO_2$ assimilation rate ($A$). Activity of antioxidant enzymes was increased by drought stress significantly. Amounts of malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$) remained unchanged in Se-supplemented water-deficit plants obviously because of an efficient scavenging following significant enhancement of catalase (CAT) and glutathione peroxidase (GSH-Px) activities. These results indicate that an application of selenium was favorable for biomass accumulation of barley plants under well-watered conditions. However, it did not significantly affect dry matter accumulation under drought stress, but Se-supplemented water-deficit plants exhibited better protection from oxidative damage because of higher CAT and GSH-Px activities and lower level of lipid peroxidation. These results suggest that selenium application can improve antioxidant defense system under drought stress conditions, and it may be recommended for arid and semiarid regions.

**Key words:** Antioxidant enzymes, barley, drought, glutathione peroxidase, selenium

**IZVLEČEK**

**UČINEK SUŠNEGA STRESA IN ŠKROPLJENJA S SELENOM NA FOTOSINTEZO IN ANTIOKSIDATIVNO AKTIVNOST JAREGA JEČMENA**

Članek poroča o učinku škropljenja s selenom na nekatere fiziološke značilnosti ječmena (*Hordeum vulgare* 'Rihane-03'), ki je bil izpostavljen sušnemu stresu. Foliarna aplikacija selena 30 g Se $ha^{-1}$, kot selenat je značilno povečala suho težo poganjkov in relativno vsebnost vode dobro zalivanih rastlin. Znatno zmanjšanje suhe teže rastlin v pomanjkanju vode je bilo povezano z značilnim zmanjšanjem maksimalne učinkovitosti PSII ($F_v/F_m$), stomatarne prevodnosti ($g_s$) in neto asimilacije $CO_2$ ($A$). Aktivnost antioksidativnih encimov se je v sušnem stresu značilno povečala. Količini malondialdehida (MDA) in vodikovega peroksida ($H_2O_2$) sta ostali nespremenjeni pri rastlinah tretiranih s Se pri sušnem stresu, kar je bila očitno posledica delovanja Se, ki se je kazala kot povečana aktivnost katalaze (CAT) in glutation peroksidaze (GSH-Px). Ti izsledki kažejo, da uporaba Se prispeva k povečanju biomase ječmena pri dobri preskrbi z vodo. Vsebnost suhe snovi se pri rastlinah tretiranih s Se ni bistveno povečala v razmerah sušnega stresa, vendar pa so se te rastline bolje zaščitile pred oksidativnimi poškodbami s povečano aktivnostjo CAT in GSH-Px in manjšo peroksidacijo lipidov. Rezultati kažejo, da uporaba Se izboljša antioksidativno obrambo rastlin pri sušnem stresu in bi njegovo uporabo v te namene priporočali v aridnih in semiaridnih območjih.

**Ključne besede:** antioksidativni encimi, ječmen, suša, glutation peroksidaza, selen (Se)

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## 1 INTRODUCTION

Although selenium (Se) is not an essential element for plants (Terry *et al*., 2000), several studies demonstrate that selenium supply may exert diverse beneficial effects, including growth-promoting activities (Turakainen *et al*., 2004; Djanaguiraman *et al*., 2005). Moreover, some plant species grown in Se-enriched media have shown enhanced resistance to certain abiotic stresses, e.g. drought (Kuznetsov *et al*., 2003; Germ *et al*., 2007; Yao *et al*., 2009), salinity (Kong *et al*., 2005; Djanaguiraman *et al*., 2005; Hawrylak-Nowak, 2009) and heavy metals (Srivastava *et al*., 2009; Cartes *et al*., 2010) stresses. Selenium exerts beneficial effects on growth and stress tolerance of plants by enhancing their antioxidative capacity (Kong *et al*., 2005; Rios *et al*., 2009).

A stimulatory effect of foliar application of Se on growth has been reported for ryegrass (Hartikainen *et al*., 2000), lettuce (Xue *et al*., 2001), potato (Turakainen *et al*., 2004), soybean (Djanaguiraman *et al*., 2005) and green tea leaves (Hu *et al*., 2003). Selenium can also delay senescence and promote the growth of aging seedlings (Xue *et al*., 2001). Selenium supplemented water-deficit buckwheat exhibit significantly higher stomatal conductance ($g_s$). A significantly higher actual photochemical efficiency of PSII was obtained in Se-supplemented water-deficit plants, which was possibly due to improvement of plant water management during treatment (Tadina *et al*., 2007). Selenium supply is favorable for growth of wheat seedlings during drought condition, however, the growth and physiological responses of seedlings were different, depending on the Se concentration (Yao *et al*., 2009). Simojoki (2003) reported that small Se addition that increased Se contents in lettuce shoots tend to enhance plant growth. It was shown that Se has the ability to regulate the water status of plants under drought conditions (Kuznetsov *et al*., 2003).

Recent researches have demonstrated that Se is not only able to promote growth and development of plants, but also increases resistance and antioxidant capacity of plants subjected to various stress (Peng *et al*., 2002; Djanaguiraman *et al*., 2005). Stress factors such as drought trigger common reactions in plants and lead to cellular damages mediated by reactive oxygen species (ROS). According to Price and Hendry (1991) who studied the role of oxygen radicals in different grasses exposed to drought, water deficit stress causes an overall inhibition of protein synthesis, inactivation of several chloroplast enzymes, impairment of electron transport, increased membrane permeability, and increased activity of the $H_2O_2$ scavenger system. Antioxidative enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) play an important role against drought stress (Apel and Hirt, 2004; Habibi and Hajiboland, 2011). However, there are few reports on the protective role of exogenous Se on drought stress in plant. Also, the plants treated with selenate induce higher increases in enzymes that detoxify $H_2O_2$, especially ascorbate peroxidase (APX) and glutathione peroxidase (GSH-Px), thereby improving stress resistance (Kong *et al*., 2005; Rios *et al*., 2009).

Foliar application to barley at 10 and 20 g Se $ha^{-1}$, as sodium selenate, increases the Se contents of barley grain (MacLeod *et al*., 1998). Morever, a stimulatory effect of foliar application of Se on nitrogen assimilation has been reported for barley (Aslam *et al*., 1990), however, there is few published work concerning the expression of selenium effects on dry matter accumulation, protection against the oxidative stress and regulation the water status of *Hordeum vulgare* L. (barley). Barley is one of the most important crops. It is well characterized by the multiplicity of its agro-industrial uses. The aim of present work was to study the influence of Se on the photosynthesis characteristics and antioxidant activity in spring barley. In addition of monitoring the growth, relative water content, chlorophyll fluorescence parameters and gas exchange pattern, we examined the effect of selenium spraying on the antioxidant defense system during drought stress in barley plants.

## 2 MATERIALS AND METHODS

*Plant growth and treatments*: Seeds of barley (*Hordeum vulgare* L. cv. Rihane-03) were grown in a field trial in sandy loam soil near Malekan, NW Iran. Seeds planted on 5 rows in each plots, the rows distance was 20 cm and the plant distance on each row was 5 cm, beginning and end of each plots closed, with regarding area of each plots. For the basal fertilization, 100 kg $ha^{-1}$ nitrogen as $NH_4NO_3$ and 50 kg $ha^{-1}$ phosphorus and potassium as $KH_2PO_4$ were applied before sowing. Experiments were performed in complete randomized block design with 4 replications. The replicates were separated at random into two groups; well watered group and water-stressed group. For normal irrigation (well watered group), soil was kept at approximately 70% of field capacity by watering with tap water every 7 days and water holding at the beginning of stem elongation stage in water-stressed group of plants. After 35 days of drought exposure, selenium was sprayed at 30 g $ha^{-1}$ as sodium selenate. After 10 days of selenium exposure, the plants were harvested and parameters were determined. Thousand seed weight and seed yield were measured at the end of the experiment.

*Plant harvest and 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 field.

*Measurements of photosynthetic gas exchange and chlorophyll fluorescence*: Before harvest gas exchange parameters were measured. Net $CO_2$ 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) either after 5 h into the light period and sealed in the leaf chamber under a photon flux density of 2000 ± 100 µmol $m^{-2}$ $s^{-1}$ in field 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*: Fresh samples were ground in the presence of liquid nitrogen and measurements were undertaken using spectrophotometer (Specord 200, Analytical Jena, Germany). The activity of superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11) and catalase (CAT, EC 1.11.1.6) was determined as described by Habibi and Hajiboland (2010). 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 contained 10 mM phosphate buffer, 5 mM $H_2O_2$ and 4 mM guaiacol. The glutathione peroxidase (GSH-Px, EC 1.11.1.9) activity was measured by a modification of the method of Flohé and Günzler (1984) using the $H_2O_2$ as substrate. Enzyme was extracted in 50 mM phosphate buffer pH 7.0 and the supernatant was added to the reaction mixture contained 0.2 mL of the supernatant, 0.4 mL GSH (0.1 mM) and 0.2 mL $KNaHPO_4$ (0.067 M). The above reagents without supernatant extract were used for the non-enzyme reaction. After preheating the mixture on water bath at 25 °C for 5 min, 0.2 mL $H_2O_2$ (1.3 mM) was added to initiate the reaction. The reaction was stopped by adding 1 mL 1% trichloroacetic acid and the mixture was put into an ice bath for 30 min. Then the mixture was centrifuged for 10 min at 1100 g, 0.48 mL the supernatant was placed into a cuvette and 2.2 mL of 0.32 M $Na_2HPO_4$ and 0.32 mL of 1.0 mM DNTB were added for colour development. The absorbance at wavelength 412 nm was measured after 5 min. The enzyme activity was calculated as a decrease in GSH within the reaction time when

compared with that in the nonenzyme reaction. Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid, and the $H_2O_2$ concentration was determined by the potassium titanium oxalate method (Hajiboland and Hasani, 2007). Soluble protein was estimated spectrophotometrically by the Bradford method (1976). Statistical analyses were 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 measurement parameters.

## 3 RESULTS

Both relative water content (RWC) and dry weight decreased dramatically in water-stressed plants. In contrast to drought, selenium spraying treatment increased relative water content and dry matter accumulation in well-watered plants, as compared with control plants (Table 1). Thus, the treatment with the highest dry matter accumulation (Se-supplemented well-watered treatment) showed the highest relative water content (83.5%). At the end of the experiment, Thousand seed weight and seed yield decreased by 23.4 and 49.6% under water stress, respectively, in comparison to their respective plants under well-watered conditions. Seed yield was not affected by selenium spraying treatment.

**Table 1:** Shoot dry weight (mg plant$^{-1}$), thousand seed weight (g), seed yield (kg ha$^{-1}$) and leaf relative water content (RWC, %) under different treatments. Each value is the mean ± SD of 20 replicates. Data of each column indicated by the same letters are not significantly different ($P < 0.05$).

| Treatments | Shoot dry weight | Thousand seed weight | Seed yield | Relative water content |
|---|---|---|---|---|
| Control | 1845 ± 131 [b] | 47.7 ± 4.19 [a] | 2887 ± 141 [a] | 72.5 ± 2.38 [b] |
| Drought | 1120 ± 120 [c] | 36.5 ± 4.43 [b] | 1455 ± 161 [b] | 55.2 ± 3.11 [c] |
| Selenium | 2100 ± 212 [a] | 51.2 ± 2.38 [a] | 2995 ± 174 [a] | 83.5 ± 3.69 [a] |
| Drought+Selenium | 1210 ± 143 [c] | 37.2 ± 2.50 [b] | 1565 ± 83 [b] | 57.6 ± 2.64 [c] |

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 Se-supplemented plants under well-watered conditions (Table 2). However, reduction of maximal efficiency of PSII in dark-adapted leaves ($F_v/F_m$) and effective quantum yield of PSII ($\Phi_{PSII}$) were detectable in leaves of water-stressed plants. In addition, stomatal conductance to water vapor ($g_s$) was positively correlated with $F_v/F_m$ ($r = 0.70$, $P < 0.05$) in water-stressed plants. Photochemical quenching ($qP$) and non-photochemical quenching ($qN$) were not influenced under selenium spraying and drought conditions.

Net assimilation rate ($A$) was not influenced by selenium spraying, but was reduced by drought (Table 2). Transpiration rate ($E$) was not affected significantly by water stress, while $g_s$ was reduced strongly under drought conditions but increased by selenium. In this study, a remarkable reduction in shoot dry weight in drought stressed plants was associated with a significant reduction of net $CO_2$ assimilation rate. 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 the water deficit.

**Table 2:** Leaf physiological traits of barley plants under different treatments. *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, $\Phi_{PSII}$ 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 | Control | Drought | Selenium | Drought+Selenium |
|---|---|---|---|---|
| $F_v/F_m$ | 0.84 ± 0.01[a] | 0.81 ± 0.01[b] | 0.84 ± 0.02[a] | 0.82 ± 0.01[ab] |
| *qP* | 0.96 ± 0.02[a] | 0.96 ± 0.02[a] | 0.95 ± 0.02[a] | 0.95 ± 0.01[a] |
| *qN* | 0.17 ± 0.05[a] | 0.15 ± 0.02[a] | 0.14 ± 0.09[a] | 0.16 ± 0.08[a] |
| $\Phi_{PSII}$ | 0.79 ± 0.01[a] | 0.76 ± 0.01[b] | 0.79 ± 0.01[a] | 0.76 ± 0.01[b] |
| **Gas exchange** | **Control** | **Drought** | **Selenium** | **Drought+Selenium** |
| *A* (µmol $m^{-2}$ $s^{-1}$) | 14.2 ± 3.52[a] | 5.17 ± 1.63[b] | 16.3 ± 1.46[a] | 6.93 ± 2.40[b] |
| *E* (mmol $m^{-2}$ $s^{-1}$) | 5.95 ± 0.67[a] | 3.61 ± 1.34[a] | 5.54 ± 0.14[a] | 4.65 ± 1.40[a] |
| $g_s$ (mol $m^{-2}$ $s^{-1}$) | 0.41 ± 0.05[ab] | 0.27 ± 0.13[b] | 0.52 ± 0.13[a] | 0.36 ± 0.10[ab] |
| *WUE* (µmol $mmol^{-1}$) | 2.39 ± 0.59[a] | 1.44 ± 0.07[b] | 2.94 ± 0.16[a] | 1.49 ± 0.12[b] |

Activity of antioxidant enzymes were influenced by drought stress significantly (Table 3). Drought stress caused a significant increase of SOD, POD, CAT and APX activity relative to control plants. In contrast, activity of GSH-Px was only increased in Se-supplemented water-deficit plants. Activity of SOD and APX in water-stressed plants did not differ from that in Se-supplemented water-deficit plants. However, a significant rise in the activity of GSH-Px and CAT was observed in the Se-supplemented water-deficit samples relative to water-deficit treatment. Continuation of the water stress without selenium spraying caused significant accumulation of malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$) content. In contrast to drought stressed plants, in Se-supplemented water-deficit plants, the content of MDA and $H_2O_2$ remained quite stable over experimental period. There was a good negative correlation ($r = -0.69$, $P < 0.05$ in selenium treatment; $r = -0.64$, $P < 0.05$ in drought+selenium treatment) between GSH-Px activity and MDA content. Thus, the malondialdehyde content of leaves was highly associated with the glutathione peroxidase activity under selenium application.

**Table 3:** Antioxidant index of barley plants under different treatments. SOD superoxide dismutase, CAT catalase, POD peroxidase, APX ascorbate peroxidase, GSH-Px glutathione peroxidase, MDA malondialdehyde, $H_2O_2$ hydrogen peroxide. Each value is the mean ± SD of 4 replicates. Data of each row indicated by the same letters are not significantly different ($P < 0.05$).

| Specific activity of enzymes | Control | Drought | Selenium | Drought+Selenium |
|---|---|---|---|---|
| SOD (U $mg^{-1}$ protein) | 11.1 ± 0.52[b] | 21.1± 2.84[a] | 15.1 ± 0.54[b] | 26.3 ± 4.15[a] |
| CAT (µmol $mg^{-1}$ protein $min^{-1}$) | 44.2 ± 6.0[c] | 82.1 ± 16.3[b] | 52.2 ± 8.1[c] | 127 ± 10.6[a] |
| POD (µmol $mg^{-1}$ protein $min^{-1}$) | 0.27 ± 0.07[b] | 0.49 ± 0.01[a] | 0.31 ± 0.02[b] | 0.52 ± 0.01[a] |
| APX (µmol $mg^{-1}$ protein $min^{-1}$) | 0.89 ± 0.04[b] | 1.30 ± 0.14[a] | 0.82 ± 0.03[b] | 1.26 ± 0.12[a] |
| GSH-Px (nmol $mg^{-1}$ protein $min^{-1}$) | 0.10 ± 0.01[c] | 0.14 ± 0.02[bc] | 0.16 ± 0.03[b] | 0.24 ± 0.01[a] |
| **Metabolite content** | **Control** | **Drought** | **Selenium** | **Drought+Selenium** |
| MDA (nmol $g^{-1}$ FW) | 14.7 ± 0.50[b] | 29.8 ± 1.20[a] | 12.9 ± 1.43[b] | 16.7 ± 0.93[b] |
| $H_2O_2$ (µmol $g^{-1}$ FW) | 0.84 ± 0.10[b] | 1.15 ± 0.12[a] | 0.86 ± 0.04[b] | 0.93 ± 0.08[b] |

## 4 DISCUSSION

Most probably the first positive effect of selenium on plant growth was reported by Singh *et al.* (1980), who showed that the application of selenium stimulated growth and dry matter yield of *Brassica juncea*. In this work, similarly with that observed for lettuce, ryegrass (Hartikainen *et al.*,

1997; Hartikainen *et al*., 2000) and soybean (Djanaguiraman *et al*., 2005), selenium increased shoot dry weight in barley plants. Drought stress reduced growth activity of barley (Tables 1), as is observed by other plants species (Ramesh, 1999; Liu and Stützel, 2004; Degu *et al*., 2008). Recently, Yao *et al*. (2009) suggested that optimal Se supply is favorable for growth of wheat seedlings during drought condition. In this work, however, selenium spraying could not improve growth parameters under drought conditions.

An important role associated with the survival of the plants grown under drought conditions is played by the leaf stomata. In the present study, the stomatal density decreased significantly with water stress. Reduction of stomatal conductance ($g_s$) inhibits supply of $CO_2$ and consequently reduces $CO_2$ assimilation ($A$) is a well known phenomenon in drought stressed plants (Lawlor and Cornic, 2002). Our results confirm the great sensitivity of leaf photosynthesis to drought. 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). Following the drought stress, Se-supplemented plants showed a lower reduction in these photosynthetic parameters ($A$ and $g_s$) in response to drought stress when compared with water-deficit plants. Thus, similarly with that observed for buckwheat (Tadina *et al*., 2007), stomatal conductance ($g_s$) was slightly higher in Se-supplemented water-deficit plants, but net $CO_2$ assimilation rate did not increase in Se-supplemented water-deficit plants relative to water-deficit plants in this study. The water status of the barley leaves has been put in evidence by water use efficiency (WUE) and relative water content (RWC) parameters, so the RWC rate and WUE decreased significantly because of unchanged transpiration rate ($E$) at water stress in both treatments tested. On the other hands, the comparison of the leaf water content between the two well-watered treatments showed that leaf water content of selenium treatment increased more than control. The net photosynthetic rate and stomatal conductance of higher plants leaves are known to decrease as RWC decrease (Lawlor and Cornic, 2002).

Many authors suggested 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). To test the functionality of the photochemical apparatus, barley plants were treated with drought stress. We found that the significant decrease in their maximal efficiency of PSII photochemistry ($F_v/F_m$) were observed at water stress conditions. In agreement with the results of Angelopoulos *et al*. (1996) showing that during the development of water stress a gradual decline of the ratio $F_v/F_m$ occurred. Recently, Boussadia *et al*. (2008) showed that $F_v/F_m$ was reduced significantly in plants submitted to water deficit. Declining $F_v/F_m$ values implies that photochemical conversion efficiency is altered and could indicate the possibility of photoinhibition (Ranjbarfordoei *et al*., 2006). In our study, photoinhibition was occurred in drought treatment. A reduction in maximum quantum yield of PSII ($F_v/F_m$) was obtained in water-deficit plants (Table 2), which was possibly due to the reduction of $g_s$ and restriction of $CO_2$ for photosynthesis and indicated photoinhibition. The significant correlation between $F_v/F_m$ and $g_s$ confirmed the idea that limited carbon assimilation by the decrease in stomatal conductance is viewed as an important protective mechanism under drought. This positive correlation suggests that the reduction in maximal efficiency of PSII photochemistry particularly at drought stress may be due to factors affecting stomatal closure rather than to damages in the photosynthetic apparatus. In addition, increasing stomatal conductance to water vapor resulted in subsequent increase in the net photosynthetic rate under selenium spraying showed that stomata are the main limiting factor to carbon uptake (Cornic *et al*., 1992; Boussadia *et al*., 2008).

Plants protect cell systems from the cytotoxic effects of drought-accumulated active oxygen species using enzymes such as SOD, APX, glutathione peroxidase (GSH-Px) and CAT (Verhagen *et al*., 2004). Several studies have shown that a protective role of Se against the oxidative stress in higher plants coincided with enhanced GPX activity and decreased lipid peroxidation (Cartes *et al*., 2005). Selenate application at 30 g $ha^{-1}$ caused variations in the

SOD, APX and POD activity and also increased the CAT and GSH-Px activities (Table 3). The results for GSH-Px coincide with several studies made with Se in which an increase in this trace element augmented its activity (Xue *et al*., 2001; Cartes *et al*., 2005). In this work, a significant rise in the activity of GSH-Px and CAT in the Se-supplemented water-deficit samples relative to water-deficit treatment revealed that Se exerts beneficial effects on stress tolerance of barely by enhancing their antioxidative capacity. Amounts of MDA and $H_2O_2$ remained unchanged under Se-supplemented water-deficit conditions obviously because of an efficient scavenging following significant enhancement of CAT and GSH-Px activity. It indicate that antioxidant defense system may protect plants under Se-supplemented water-deficit conditions, while under water stress without selenium spraying, an imbalance between production and scavenging of ROS may cause stress as could be judged by accumulation of MDA.

In summary, we investigate the changes in physiological parameters in spring barley grown under drought stress and selenium spraying. Our results show that selenium spraying (1) causes a significantly higher growth rate, (2) affects plant water relations, as expressed by a significant decrease in leaf water content and (3) causes a significant increase in antioxidative capacity under drought conditions. Physiological and molecular mechanisms that underlie the beneficial effects of Se in plants have not been fully explained yet. There are few reports on the protective role of exogenous Se on drought stress in plant. In conclusion, Se-supplemented water-deficit plants exhibited better protection from oxidative damage and this ability was associated with higher CAT and GSH-Px activities and lower level of lipid peroxidation. These data indicate that an application of selenate at low rates can be used to promote the induction in plants of the antioxidant system, thereby improving stress resistance. However, it would be necessary to confirm these results in future with studies that focus on the effect of Se during the application time and the response of the different isoenzymes that make up the antioxidant system in plants. From this conclusion we can say that Se treatment did not significantly affect dry mass under drought conditions, although it increased dry matter in well watered plants and some antioxidant index in water-deficit plants. These results suggest that selenium application can improve antioxidant defense system under drought stress conditions, and it may be recommended for soils in arid and semiarid regions.

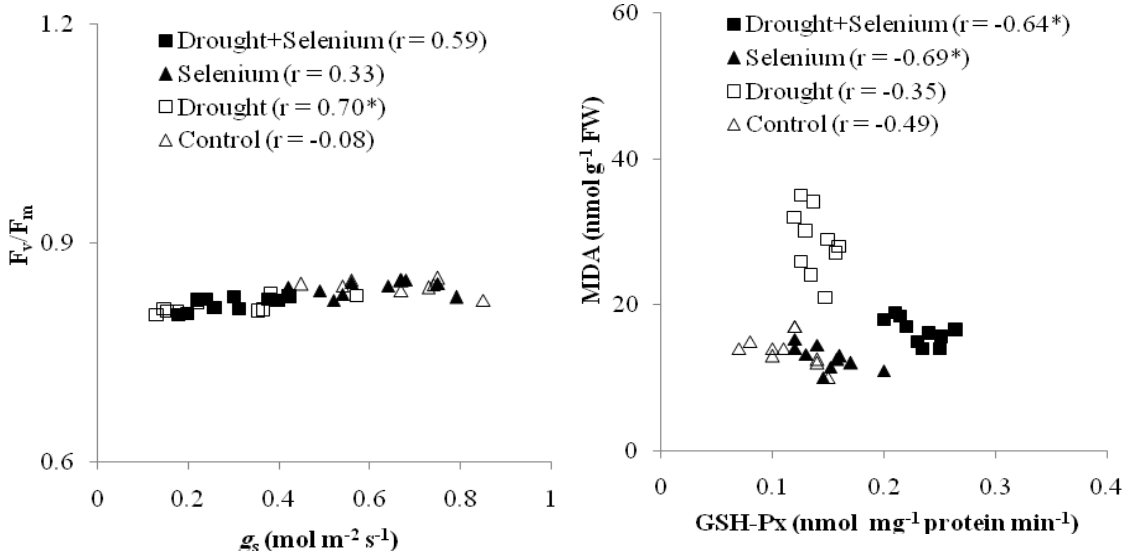


**Figure 1**: Correlations between maximum quantum yield of PSII ($F_v/F_m$) and stomatal conductance ($g_s$) and between glutathione peroxidase (GSH-Px) activity and malondialdehyde (MDA) content in barley plants at different treatments. ns, *, and **: non-significant and significant, at the 5% and 1% levels of probability, respectively.

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