

DOI: 10.14720/aas.2014.103.2.2

Agrovoc descriptors: triticum durum, hard wheat, genotypes, photosynthesis, proline, amino acids, drought stress, water use, efficiency, plant water relations, selenium, osmosis, watering**Agris category code:** f60, f06

Effect of Se application on photosynthesis, osmolytes and water relations in two durum wheat (*Triticum durum* L.) genotypes under drought stress

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Received May 29, 2014; accepted August 01, 2014.

Delo je prispelo 29. maja 2014, sprejeto 01. avgusta 2014.

ABSTRACT

Effect of Se (as Na₂SeO₄ at final concentration of 10 µg l⁻¹) was studied in two durum wheat (*Triticum durum* L.) genotypes in perlite under drought conditions. Se treatment increased slightly biomass of both genotypes under drought but not under control conditions. Photosynthetic rate was depressed by drought while increased by Se treatments in both genotypes up to 2.3 fold. However, transpirational water loss was also enhanced in Se-treated plants under both well-watered and drought conditions. Se application resulted in higher concentrations of soluble proteins and free α-amino acids under drought conditions, but not proline. Our results indicated that Se application improves some physiological parameters such as photosynthesis, accumulation of osmolytes and water use efficiency but did not change significantly plants biomass or water relation parameters.

Key words: organic osmolytes, photosynthetic rate, proline, water use efficiency, water relations

IZVLEČEK

UČINEK UPORABE Se NA FOTOSINTEZO, VSEBNOST OSMOTIKOV IN VODNI REŽIM PRI DVEH GENOTIPIH TRDE PŠENICE (*Triticum durum* L.) V RAZMERAH SUŠNEGA STRESA

Preučevani so bili učinki Se, dodanega kot Na₂SeO₄ v koncentraciji 10 µg l⁻¹ na dveh genotipih trde pšenice (*Triticum durum* L.) gojene v perlitu v razmerah sušnega stresa. Tretiranje s Se je neznatno povečalo biomaso obeh genotipov v razmerah sušnega stresa, a ne pri kontroli. Fotosinteza je upadla s sušo, a se je po tretiranju s Se pri obeh genotipih povečala za 2.3 krat. Transpiracijska izguba vode je bila pospešena pri rastlinah tretiranih s Se v razmerah sušnega stresa kot pri dobro zalitih rastlinah. Uporaba Se se je odrazila v večji koncentraciji topnih beljakovin in prostih α-amino kislin v razmerah suše, vendar ne v koncentraciji prolina. Naši rezultati kažejo, da uporaba Se izboljša nekatere fiziološke parameter kot so fotosinteza, akumulacija osmotikov in učinkovitost izrabe vode, vendar ne spremeni značilno biomase rastlin in parametrov vodnega režima rastline.

Ključne besede: organski osmotiki, fotosinteza, prolin, učinkovitost izrabe vode, vodni režim

1 INTRODUCTION

Plants often encounter unfavorable environmental conditions that limit their growth and productivity. Among the various abiotic stresses, drought is the major factor that reduces crop yields worldwide (Farooq et al., 2009). Drought stress leads to a

series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity (Yordanov et al., 2003; Chaves et al., 2009).

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** This paper is a part of the M.Sc. thesis of N.S. under supervision of R.H. and B.S.

Stomatal closure is one of the earliest responses of plants to water deficit that limits transpirational water loss and helps plants to retain water status under drought. However, closure of stomata in turn, results in reduction of CO₂ availability for photosynthetic carbon metabolism, depresses net CO₂ assimilation rate and inhibits plants ability for dry matter accumulation (Chaves et al., 2009). In addition, declines in the CO₂ availability to the Calvin cycle enzymes result in lower regeneration of NADP⁺ and production of excess excitation energy that damages photosystems (Hajiboland, 2014).

Synthesis and accumulation of organic osmolytes as compatible solutes is one of the mechanisms for adaptation to water deficit. Accumulation of osmoprotectants including free amino acids, particularly proline and soluble carbohydrates, improves water extraction capacity from dry substrate (Morgan, 1984). There are also numerous evidences on the effect of these compounds in protecting membranes and cell structures from damages caused by dehydration and scavenging of free radicals (Verbruggen and Hermans, 2008).

Selenium is not yet considered an essential nutrient for higher plants and is toxic at higher concentrations (Hartikainen et al., 2000; Germ and Stibilj, 2007). Research works on plants under various environmental conditions showed that Se ameliorates adverse effects of stress (Hajiboland, 2012; Feng et al., 2013). However, the possible mechanisms for the Se-enhanced tolerance of plants to environmental stress have not been fully elucidated (Feng et al., 2013).

Researches on the alleviating effect of Se on environmental stress have mainly focused on the Se-mediated activation of antioxidative defense (Feng et al., 2013). Considering plants biomass, however, a correlation has not been found between the activity of antioxidative defense enzymes and the extent to which Se influenced growth

(Hajiboland and Amjad, 2007) and alleviated stress (Yao et al., 2009, Habibi, 2013). Under drought conditions, important parameters such as accumulation of osmotic substances and water uptake capacity as affected by Se are largely unstudied. Se application increased the accumulation of proline (Yao et al., 2009). However, it did not influence water uptake capacity and biomass of plants under drought (Yao et al., 2009; Habibi, 2013). On the other hand, effect of Se on the improvement of plants growth is not restricted to stressful conditions and its application affects positively plants growth and reproduction under optimum growth conditions (Hajiboland and Amjad, 2007; Hajiboland and Keivanfar, 2012). Recently we reported higher CO₂ and NO₃⁻ assimilation in Se-supplemented wheat plants grown hydroponically under low or adequate nitrogen supply (Hajiboland and Sadeghzadeh, 2014) that evidenced marked alterations in the N and C metabolism by Se.

Research works on the effect of Se on graminaceous crops have been reported for wheat (Yao et al., 2009) and barley (Habibi, 2013), but effect of Se on the amelioration of drought stress in durum wheat has not been studied so far. Durum is mainly grown under rainfed conditions characterized by low and highly variable rainfall and/or extreme temperatures with high probability of terminal drought and heat stress (Nsarellah et al., 2000). In Iran, durum wheat occupies about 10% of total wheat production areas, where drought is main limiting factor for crop production. Durum breeders therefore need to adopt new strategies to alleviate drought stress (Mohammadi et al., 2014). This work was undertaken in order to investigate the effects of Se application on growth, photosynthesis and water relation parameters in two contrastive durum wheat genotypes. This work will provide further information on the mechanisms involved in the Se-improved drought tolerance in graminaceous crops.

2 MATERIALS AND METHODS

Plant culture and treatments

Seeds of two durum wheat genotypes (*Triticum turgidum* ssp. durum L. 'Saji' and 'Eradyt-89')

provided by Dryland Agricultural Research Institute (DARI) (Maragheh, Iran) were surface-sterilized and germinated in dark on perlite. According to the instruction of providing institute,

'Saji' is more tolerant genotype to drought stress compared with 'Erduyt-89' (B. Sadeghzadeh, personal communication). After 3 days, seedlings were transferred into light. Five-days-old seedlings were transferred into 1.5 L pots filled with acid-washed perlite irrigated with water or 50% wheat nutrient solution with the following composition (mM): 2.0 Ca (NO₃)₂, 1.0 MgSO₄, 0.9 K₂SO₄, 0.25 KH₂PO₄, 0.1 KCl and (μM): 2.0 H₃BO₃, 0.4 MnSO₄, 1.0 ZnSO₄, 0.4 CuSO₄, 0.1 FeEDTA and 0.04 (NH₄)₆MO₇O₂₄ (Hajiboland et al., 2003). Forty plants were cultured in each pot and each treatment and cultivar was represented by 4 replicate pots. Se treatments at two levels including without (-Se) or with (+Se) Se and irrigation treatments including well-watered (irrigation at field capacity) and drought stress (irrigation at 30 % FC) were started simultaneously 9 days after transplanting. Se and irrigation treatments were assigned randomly to the pots. Application of Se (as Na₂SeO₄) was performed gradually during 4 weeks with the total final amount of 10 μg l⁻¹. Drought stress was imposed by omitting watering and after one week, pots reached the 30% FC. Throughout the experiment, pots were irrigated daily after weighing with nutrient solution or water as interval. Control and water-stressed plants received the same amount of nutrient solution and the respective FC was achieved by different volumes of water.

Plants were grown under greenhouse conditions with a day/night temperature regime of 25-28/15-17 °C, a relative humidity of 70/80% and a photoperiod of 17/7 h at a photon flux density of about 300 μmol m⁻²s⁻¹ provided by natural light supplemented with fluorescent lamps.

Plants harvest

Five weeks after starting Se treatments (4 weeks after reaching the respective FC, 7 weeks after sowing) plants were harvested. Shoot and roots were separated and after determination of fresh weight (FW), washed with distilled water and blotted dry on filter paper. Plants dry weight (DW) was determined after drying in 60 °C for 48 h. Subsamples were taken for biochemical analyses before drying. Before harvest, chlorophyll fluorescence and gas exchange parameters were determined in attached leaves.

Determination of chlorophyll fluorescence and gas exchange parameters

Maximum quantum yield of PSII (F_v/F_m) were recorded using a portable fluorometer (OSF1, ADC Bioscientific Ltd., UK). CO₂ assimilation and transpiration rates were measured with a calibrated portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK) between 10:00 A.M. and 13:00 P.M. at harvest. The measurements were conducted with photosynthetically active radiation (PAR) intensity at the leaf surface of 400 μmol m⁻² s⁻¹. The net photosynthesis rate by unit of leaf area (A , μmol CO₂ m⁻² s⁻¹), transpiration rate (E , mmol H₂O m⁻² s⁻¹) and the stomatal conductance to water vapor (g_s , mol m⁻² s⁻¹) were calculated using the values of CO₂ and humidity variation inside the chamber, both measured by the infrared gas analyzer of the photosynthesis system. Instant water use efficiency ($iWUE$) was calculated as the ratio of photosynthesis: transpiration (μmol mmol⁻¹).

Assay of leaf pigments and metabolites were performed in fresh samples after grinding at 4 °C using mortar and pestle and measurements were undertaken using spectrophotometer (Specord 200, Analytical Jena, Germany). 105 leaf explants were prepared and for plasmid pART27 2mgfp5-ER 103 explants.

Determinations of leaf pigments

Leaf concentration of Chl a, b and carotenoids (Car) were determined according to Lichtenthaler and Wellburn (1983). Leaves were homogenized in 80% cold acetone in the dark at 4 °C. After 24 h, the absorption of samples was determined at 663 (Chl a), 646 (Chl b) and 470 (Car) nm using spectrophotometer. Determination of anthocyanins was performed using a pH differential method at pH 1 and pH 4.5 in the methanol/HCl (98:2, v/v) extract and was expressed as mg of cyanidine-3-glucoside g⁻¹ FW (Giusti and Wrolstad, 2001). Total flavonoids content was determined in the methanol extract of leaves using AlCl₃-metanol (2%, w/v) as indicator at 510 nm and quercetin (Sigma) as standard (Grayer, 1989).

Determination of osmotic potential and relative water content

Osmotic potential was determined in the leaf and root samples harvested at 1 h after the lights were

turned on in the growth chamber. Samples were homogenized in pre-chilled mortar and pestle and centrifuged at 4000 g for 20 min at 4 °C. The osmotic pressure of the samples was measured by an osmometer (Heman Roebing Messtechnik, Germany), and the mosmol Kg⁻¹ data were recalculated to Mpa using the formula: osmotic potential (MPa) = -c (mosmol Kg⁻¹) × 2.5 × 10⁻³ according to the Van't Hoff equation.

Relative water content (RWC%) was measured in the leaves harvested 1 h after starting the light period and calculated according to the formula: (FW-DW)/(TW-DW) × 100. For determination of turgid weight (TW), leaf disks (5mm diameter) were submerged for 18 h in distilled water, thereafter, they were blotted dry gently on a paper towel and weighed.

Determinations of organic solutes

For determination of non-structural carbohydrates, samples were homogenized in 100 mM phosphate buffer (pH 7.5) at 4 °C, after centrifugation at 12000 g for 15 min, supernatant was used for determination of total soluble sugars whereas the pellets were kept for starch analysis (Yemm and Willis, 1954). An aliquot of the supernatant was mixed with anthrone-sulfuric acid reagent and incubated for 10 min at 100 °C. After cooling, the absorbance was determined at 625 nm. Standard curve was created using glucose (Merck). For

determination of starch, the pellet was resuspended in a 4:1 (v/v) mixture of 8 N HCl/dimethylsulfoxide (Merck). Starch was dissolved for 30 min at 60 °C under agitation. After centrifugation, the supernatant was mixed with iodine-HCl solution and after 15 min at room temperature the absorbance was determined at 600 nm. Starch (Merck) was used for the production of standard curve.

Total soluble proteins were determined using a commercial reagent (Bradford reagent, Sigma) and bovine albumin serum (BSA) as standard. Content of total free α-amino acids was assayed using a ninhydrin colorimetric method (Yemm and Cocking, 1955). Glycine was used for standard curve. For determination of proline, samples were homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged at 3000 g for 20 min. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h, and then absorbance at 520 nm was determined. Proline (Sigma) was used for production of a standard curve (Bates et al., 1973).

Experimental design and statistical analyses

The experiment was undertaken in randomized block design with four replications as four independent pots. Differences between the means were detected according to Tukey's test ($p < 0.05$) using Sigma Stat 2.03 software.

3 RESULTS

Shoot fresh weight was decreased by drought stress in -Se plants by about 51% and 57% in 'Saji' and 'Eradyt-89', respectively. Reduction of root fresh weight, however, was lower, e.g. 25% and 28% in 'Saji' and 'Eradyt-89', respectively. Shoot dry weight was not influenced by drought in 'Saji' while decreased up to 37% in 'Eradyt-89'. Root dry weight was not significantly affected by drought in both genotypes (Fig. 1).

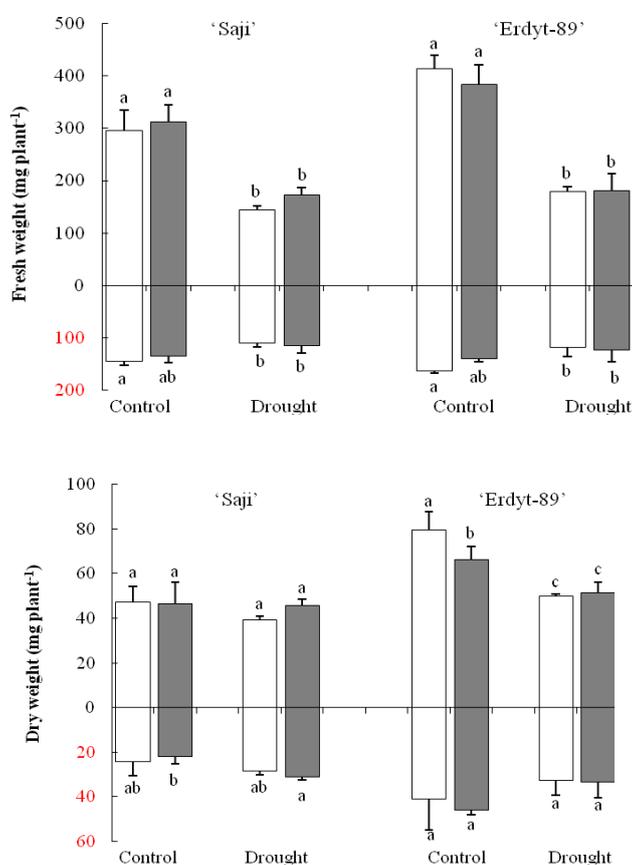
Under well-watered conditions, Se treatment did not affect positively shoot fresh or dry weight in 'Saji'. In 'Eradyt-89', it rather decreased shoot dry weight. In drought-stressed plants, fresh and dry

weight of Se-treated plants was slightly higher in 'Saji' (Fig. 1).

Leaf and root osmotic potentials were significantly lower under drought conditions in both genotypes. Se treatment, however, did not influence osmotic potentials in the leaves or roots of both genotypes. As expected, RWC was significantly lower in drought-stressed plants in both genotypes, but Se did not affect it. Instant water use efficiency was not changed in response to drought in the absence of Se. In the presence of Se, however, both genotypes showed higher water use efficiency under drought conditions compared with well-watered plants (Table 1).

Table 1: Leaf and root osmotic potential (MPa), leaf relative water content (RWC%) and instant water use efficiency (*iWUE*, the ratio of photosynthesis: transpiration) in two durum wheat genotypes grown for 4 weeks under control (100% FC) and drought (30% FC) conditions without (–Se) or with Se (+Se) ($10 \mu\text{g l}^{-1}$). Data of each column within each genotype followed by the same letter are not significantly different ($p < 0.05$).

Treatments		Osmotic potential		RWC	<i>iWUE</i>
		Leaves	Roots		
‘Saji’					
Control	–Se	-0.54 ± 0.05^a	-0.12 ± 0.03^a	81.09 ± 4.27^a	4.01 ± 0.47^b
	+Se	-0.58 ± 0.07^a	-0.12 ± 0.02^a	82.24 ± 4.46^a	3.62 ± 0.17^b
Drought	–Se	-0.74 ± 0.08^b	-0.29 ± 0.06^b	73.61 ± 4.44^b	3.62 ± 0.17^b
	+Se	-0.80 ± 0.06^b	-0.23 ± 0.04^b	70.08 ± 6.69^b	5.21 ± 0.71^a
‘Eradyt-89’					
Control	–Se	-0.51 ± 0.09^a	-0.12 ± 0.01^a	89.18 ± 2.76^a	5.22 ± 0.51^b
	+Se	-0.58 ± 0.09^a	-0.18 ± 0.05^{ab}	88.10 ± 0.42^a	5.07 ± 0.20^b
Drought	–Se	-0.78 ± 0.03^b	-0.27 ± 0.03^b	76.47 ± 2.54^b	4.79 ± 0.93^b
	+Se	-0.74 ± 0.04^b	-0.23 ± 0.02^b	79.96 ± 2.18^b	6.95 ± 1.12^a

**Figure 1:** Fresh and dry weight (mg plant^{-1}) of shoot (above of the horizontal axis) and roots (below of the horizontal axis) in two durum wheat genotypes grown for 4 weeks under control (100% FC) and drought (30% FC) conditions without (–Se, open bars) or with Se (+Se, closed bars) ($10 \mu\text{g l}^{-1}$). Bars within each genotype followed by the same letter are not significantly different ($p < 0.05$).

Drought stress did not influence concentration of leaf pigments in 'Eradyt-89' while Chl a and Car concentrations decreased under these conditions in 'Saji'. Se treatment did not change concentrations of Chl a and b in drought-stressed plants but increased these pigments significantly in well-watered ones in 'Saji'. In addition, anthocyanins and flavonoids concentrations were higher in Se-treated plants under well-watered conditions in 'Saji' while this effect in 'Eradyt-89' was observed for anthocyanins under well-watered conditions and for flavonoids in drought-stressed plants (Table 2).

Maximum efficiency of PSII (F_v/F_m) was not influenced by drought or Se in 'Saji', while a slight reduction was observed under drought stress in 'Eradyt-89'. In the latter genotype, Se-treated plants had significantly higher F_v/F_m compared with

plants without Se under drought conditions (Fig. 2A). Net photosynthetic rate was lowered significantly by drought stress in both genotypes, Se treatment increased this parameter not only in drought-stressed but also in control plants. This increase was about 37% and 60% in well-watered plants and 126% and 86% in drought-stressed ones, in 'Saji' and 'Eradyt-89', respectively (Fig. 2B). As expected, transpiration rate was lower under drought condition being significant in 'Saji' but slight in 'Eradyt-89'. Se application caused higher transpiration rate under control and drought conditions in both genotypes. However, this effect was not significant in drought-stressed 'Eradyt-89' (Fig. 2C). Similar pattern of changes was observed for stomatal conductance, however, effect of Se under drought conditions was not significant in both genotypes (Fig. 2D).

Table 2: Concentration (mg g^{-1} DW) of chlorophyll (Chl) a, b, carotenoids (Car), anthocyanins and flavonoids in the leaves of two durum wheat genotypes grown for 4 weeks under control (100% FC) and drought (30% FC) conditions without (-Se) or with Se (+Se) ($10 \mu\text{g l}^{-1}$). Data of each column within each genotype followed by the same letter are not significantly different ($p < 0.05$).

Treatments		Chl a	Chl b	Car	Anthocyanins	Flavonoids
		'Saji'				
Control	-Se	9.79±1.50 ^b	3.49±0.62 ^b	2.06±0.31 ^{ab}	51±11 ^b	12.0±1.81 ^b
	+Se	11.9±1.08 ^a	4.91±0.74 ^a	2.62±0.20 ^a	82±18 ^a	20.4±3.70 ^a
Drought	-Se	7.22±0.59 ^c	2.49±0.59 ^b	1.50±0.33 ^c	36±13 ^b	10.3±1.06 ^b
	+Se	7.70±0.27 ^c	2.62±0.34 ^b	1.63±0.15 ^{bc}	45±11 ^b	13.6±1.86 ^b
'Eradyt-89'						
Control	-Se	7.25±0.47 ^b	3.28±0.21 ^{ab}	1.93±0.10 ^{ab}	27±15 ^b	9.4±2.50 ^b
	+Se	8.05±0.64 ^{ab}	3.65±0.23 ^a	2.14±0.12 ^a	89±23 ^a	12.5±2.26 ^b
Drought	-Se	7.64±0.54 ^{ab}	3.08±0.18 ^b	1.70±0.11 ^b	20±4 ^b	9.8±1.23 ^b
	+Se	8.36±0.35 ^a	3.39±0.28 ^{ab}	1.80±0.21 ^b	43±4 ^b	16.7±1.02 ^a

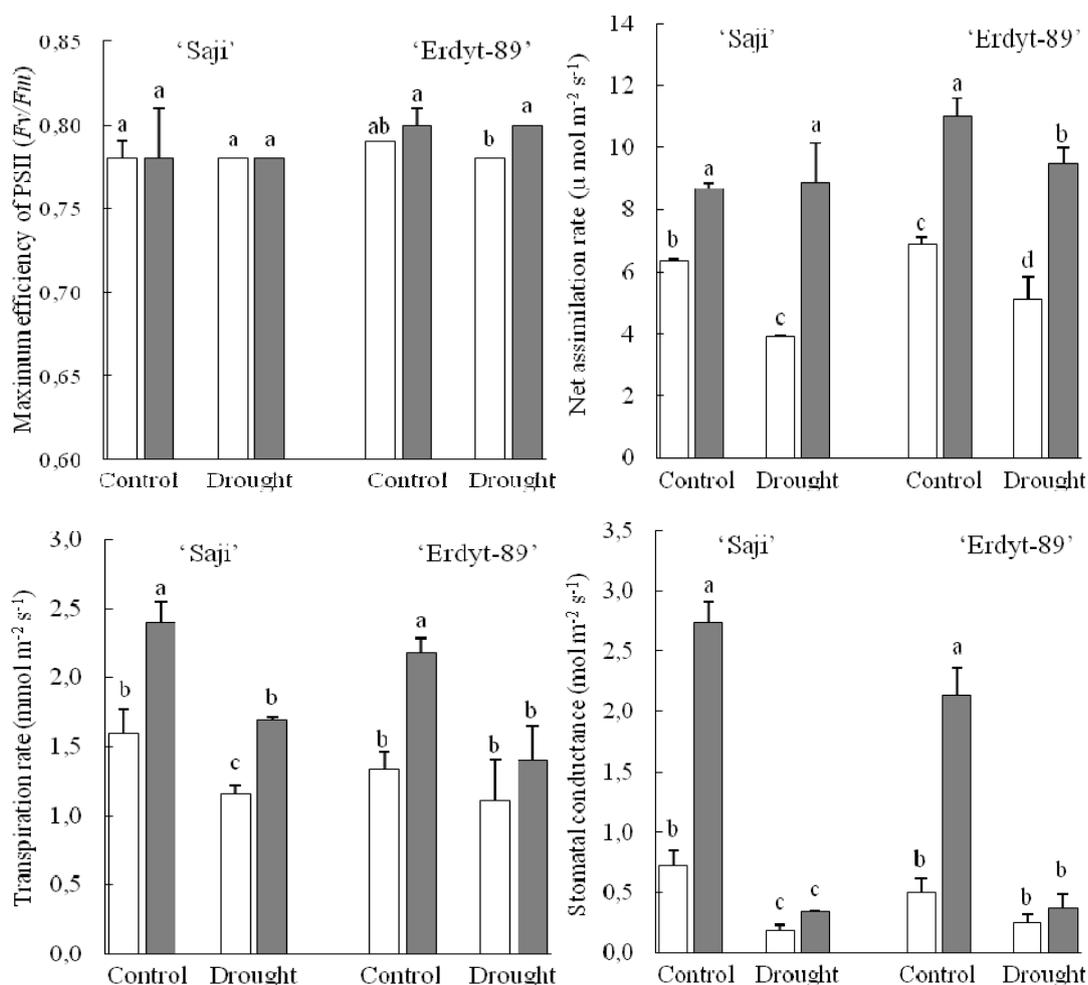


Figure 2: Maximum photochemical efficiency of PSII (F_v/F_m), net assimilation rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$), transpiration rate (E , $\text{mmol m}^{-2} \text{s}^{-1}$) and stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) in the leaves of two durum wheat genotypes grown for 4 weeks under control (100% FC), and drought (30% FC) conditions without (-Se, open bars) or with Se (+Se, closed bars) ($10 \mu\text{g l}^{-1}$). Bars within each genotype followed by the same letter are not significantly different ($p < 0.05$).

Concentration of soluble proteins in the leaves and roots was not changed by drought stress in -Se plants. In +Se plants, in contrast, drought stress decreased significantly leaf and root concentrations of soluble proteins in 'Saji'. In 'Eradyt-89', in contrast, an increase was observed in root soluble proteins in +Se plants upon drought stress. Se-treated plants, in general, had higher concentrations of soluble proteins in the leaves and roots. This effect, however, was not significant in the leaves of drought-stressed 'Saji' and in well-watered 'Eradyt-89' (Fig. 3A).

Concentration of free α -amino acids was not affected significantly by water stress conditions in -Se plants. In +Se plants, in contrast, leaf

concentration of free amino acids was slightly and significantly higher under drought conditions in 'Saji' and 'Eradyt-89', respectively. In the roots of +Se plants, drought conditions decreased free amino acids concentration in 'Saji' while increased it in 'Eradyt-89'. Se treatment caused a significant decline of leaf free α -amino acids in well-watered 'Saji' while increased it in drought-stressed plants being significant in 'Eradyt-89'. In the roots, however, Se treatment decreased free amino acids in 'Saji' under drought and in 'Eradyt-89' under well-watered conditions (Fig. 3B).

Proline accumulated in the leaves and roots of both genotypes under drought conditions. The increase in proline concentration in response to drought was

much more pronouncedly observed in the leaves of ‘Saji’. Se treatment did not affect leaf or root proline concentration in well-watered plants, while caused its reduction in the leaves of both genotypes being significant in ‘Erdyt-89’. In the roots, Se-

treated ‘Erdyt-89’ had significantly higher proline concentration under drought conditions compared with –Se plants while it was not affected by Se in ‘Saji’ (Fig. 3C).

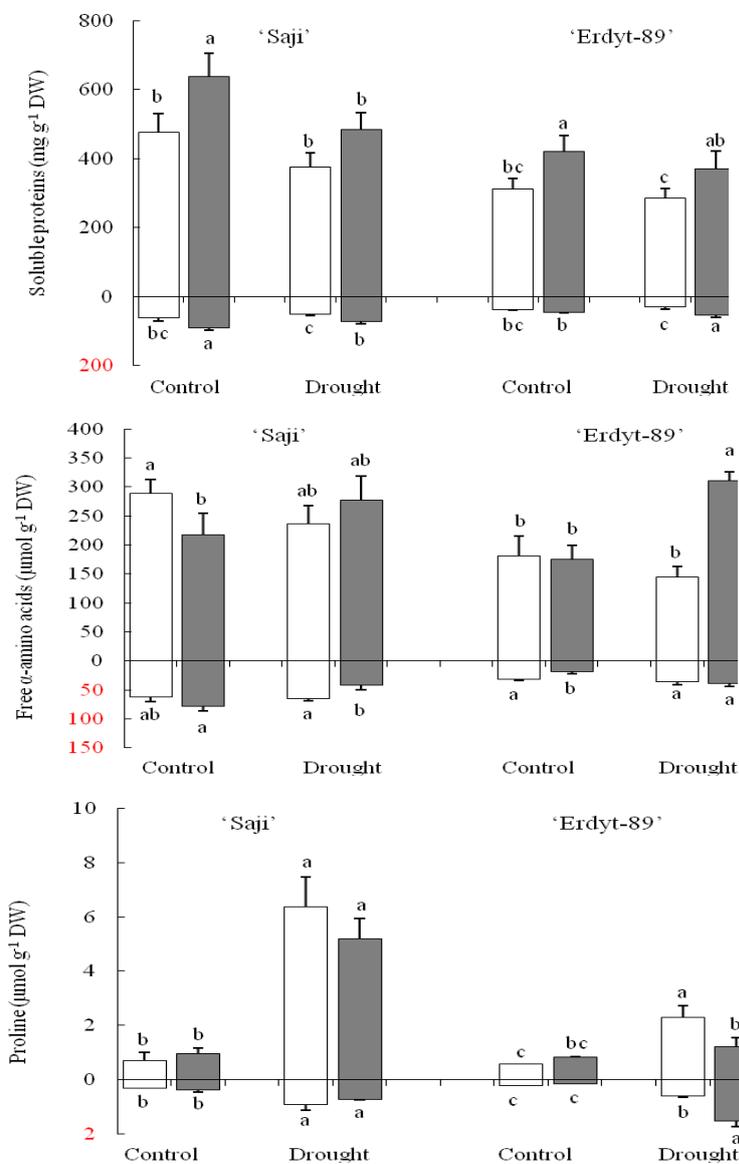


Figure 3: Concentration of soluble proteins (mg g^{-1} DW), total free α -amino acids ($\mu\text{mol g}^{-1}$ DW) and proline ($\mu\text{mol g}^{-1}$ DW) in the leaves (above of the horizontal axis) and roots (below of the horizontal axis) of two durum wheat genotypes grown for 4 weeks under control (100% FC) and drought (30% FC) conditions without (–Se, open bars) or with Se (+Se, closed bars) ($10 \mu\text{g l}^{-1}$). Bars within each genotype followed by the same letter are not significantly different ($p < 0.05$).

Soluble carbohydrates concentrations increased in response to drought in the leaves and roots. This effect, however, was not significant in the roots of

both genotypes in the absence of Se. Se-treated plants had significantly higher soluble carbohydrates in ‘Saji’ with the exception of leaves

under well-watered conditions. Effect of Se in 'Eradyt-89', however, was significant only in the roots of drought-stressed plants (Fig. 4A).

Starch concentration of leaves decreased by drought stress slightly or significantly in both

genotypes regardless of Se treatment. In the roots, in contrast, starch concentration was not affected by water deficit conditions. Se treatment did not affect significantly starch concentration of leaves and roots of both genotypes (Fig. 4B).

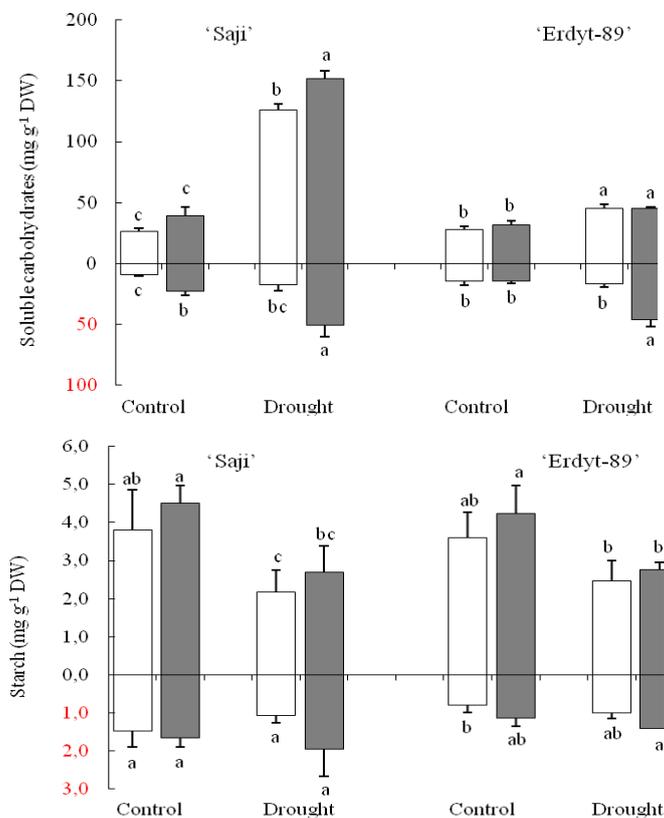


Figure 4: Concentration of soluble carbohydrates and starch (mg g⁻¹ DW) in the leaves (above of the horizontal axis) and roots (below of the horizontal axis) of two durum wheat genotypes grown for 4 weeks under control (100% FC) and drought (30% FC) conditions without (-Se, open bars) or with Se (+Se, closed bars) (10 μg l⁻¹). Bars within each genotype followed by the same letter are not significantly different ($p < 0.05$).

4 DISCUSSION

Fresh biomass of both genotypes was influenced negatively by drought stress, while dry biomass was affected by drought only in 'Eradyt-89'. It indicates higher sensitivity of 'Eradyt-89' to water deficit. This is consistent with the instruction of providing institute, but genotypic differences were higher under field condition (B. Sadeghzadeh, personal communication) compared to our greenhouse experiment.

Root biomass, in general, was much less affected by drought stress than shoot biomass leading to increase of root:shoot dry weight ratio from 0.52 in well-watered to 0.73 and 0.66 in drought-stressed 'Saji' and 'Eradyt-89', respectively. Higher root:shoot ratio is an important factor for water economy of whole-plant under water drought conditions that confer higher water uptake capacity but lower transpiration rate (Farooq et al., 2009). Se application caused only slight improvement of plants dry weight under drought conditions.

Similarly, in wheat (Yao et al., 2009) and barley (Habibi, 2013) Se did not affect dry matter production under drought conditions despite of an improvement in the antioxidative indices.

In contrast to biomass, several physiological parameters were significantly improved by Se in both durum wheat genotypes. Higher leaf concentration of pigments particularly anthocyanins and flavonoids may be important for increasing plant tolerance to various stress conditions. It has been reported that plant tissues containing anthocyanins are usually resistant to drought that is related to superoxide radical scavenging activity and of anthocyanins ability to stabilize the water potential (Chalker-Scott, 1999). Although the widely accepted antioxidant function of flavonoids in plants have been doubted by some authors (Hernández et al., 2009), their contribution to the antioxidant defense capacity of plants and its relevance in plant responses to drought have been widely accepted (Fini et al., 2011). Evidences showed that flavonoids constitute a secondary ROS-scavenging system in plants exposed to severe and prolonged stress conditions (Fini et al., 2011).

Maximum efficiency of PSII (F_v/F_m) was not influenced by drought in 'Saji' while decreased in 'Erduyt-89' that may be another indication for higher drought sensitivity in the latter genotype. Reduction of F_v/F_m revealed damage to photosystems (Hajiboland, 2014) and Se-induced recovery of this ratio implicated that Se may be effective in protection of photosynthetic apparatus from drought-induced damages via enhanced capacity of defense system (Yao et al., 2009; Habibi, 2013) or by increasing CO₂ availability for biochemical reactions i.e. higher stomatal conductance that reduces, in turn, generation of excess excitation energy (Hajiboland, 2014).

Drought conditions, as expected, reduced stomatal opening and in consequence, decreased net transpiration rates. However, 'Saji' was more efficient in the reduction of stomatal conductance and limitation of water loss than 'Erduyt-89' under drought stress. Plants respond to drought primarily by closing stomata for minimizing water loss (Yordanov et al., 2003; Chaves et al., 2009).

As the consequence of stomatal closure, CO₂ diffusion inside leaves decreases and reduction of photosynthesis impairs plants dry matter production under drought (Yordanov et al., 2003). Se treatment here elevated stomatal opening under both control and drought conditions in both genotypes and increased net assimilation rate considerably. Increase in CO₂ fixation upon Se treatment was up to 2.3 and 1.9 fold than –Se plants in 'Saji' and 'Erduyt-89', respectively. Photosynthesis rate of drought-stressed plants supplemented with Se was even exceeded that of well-watered –Si plants (Fig. 2). Sustained or rather higher photosynthetic rate under drought may be of great importance for drought tolerance in Se-treated plants in this work. Maintenance or even elevation of CO₂ fixation under drought not only prevents NADP⁺ shortage and generation of reactive oxygen species but also provides C skeletons for synthesis of organic osmolytes that are needed particularly under drought conditions.

Higher protein content of Se-treated plants is likely resulted from increased protein synthesis. We observed previously higher nitrate reductase activity that was accompanied by higher amino acids and protein concentrations in Se-treated wheat (Hajiboland and Sadeghzadeh, 2014) and rapeseed plants (Hajiboland and Keivanfar, 2012). Soluble proteins may be involved in protection of tissues against dehydration damages (Verbruggen and Hermans, 2008) particularly in the leaves that was more affected by Se than the roots.

Concentrations of proline were increased by drought conditions in the leaves and roots of both genotypes. Nitrogenous compounds such as free amino acids, proline, glycine betaine and polyamines play important roles in the maintenance of water uptake capacity from dry substrate and protection of tissues from damages caused by dehydration (Morgan, 1984; Verbruggen and Hermans, 2008). Se treatment did not increase proline concentration, in contrast, decreased it in the leaves of both genotypes slightly or significantly. Lower proline content in Se-treated plants, implied likely that they were less affected by osmotic stress and thus, needed lower proline concentrations compared with –Se counterparts. It has been proposed that accumulation of proline in plants grown under osmotic stress is due to hyper-osmosis injury and is not associated necessarily

with higher tolerance (Lutts et al., 1999). Drought-stressed wheat plants (Yao et al., 2009), in contrast, had higher proline concentration when exposed to Se. Here Se-treated 'Erdyt-89', however, had significantly higher proline concentration in the roots under drought.

Leaf and root soluble carbohydrate concentrations were increased significantly by drought that was more pronouncedly observed in 'Saji'. In contrast, leaf starch content was lowered by water deficit. Drought-induced accumulation of soluble sugars is a well-known response in plants that is either the result of increased partitioning of photoassimilates to the synthesis of free sugars and/or enhanced starch degradation (Lee et al., 2008). Here the changes in the concentration patterns of non-structural carbohydrates in the leaves may indicate that under drought conditions soluble sugars accumulated at the expense of starch. It has been also reported that accumulated soluble sugars in white clover under drought were mainly derived from degradation of stored starch rather than a result of *de novo* synthesis (Lee et al., 2008). Se treatment increased concentration of soluble sugars in both leaves and roots of well-watered and drought-stressed plants particularly in 'Saji'. It may be primarily attributed to the higher photosynthetic rate and C assimilation. This suggestion could be confirmed by slightly or significantly higher starch concentration of Se-treated plants. This excludes, in turn, the contribution of starch degradation to the increased concentration of soluble sugars upon Se

application in contrast to that observed under drought conditions. Free soluble carbohydrates are effective compounds in osmotic homeostasis, protection of membranes and cell structures against dehydration and have free radicals scavenging activity (Niedzwiedz-Siegien et al., 2004).

Leaf and root osmotic potentials were expectedly lower under drought stress and RWC was lower in both genotypes. Se treatment did not influence these parameters significantly either under well-watered or drought conditions. It implied likely that Se treatment did not influence plants ability for water uptake that contrasted with the report of Kuznetsov (et al., 2003) on soil-grown wheat. Such discrepancy is likely related to species difference, different Se form (selenite versus selenate) and concentrations and/or different growth substrates.

Despite of lower stomatal conductance and transpiration rate in drought-stressed plants, greater reduction of photosynthetic rate caused slightly lower water use efficiency in both genotypes under drought conditions. Se treatment increased stomatal opening being significant under well-watered conditions in both genotypes. Consequently, Se-treated plants had higher transpiration rate that was significant in both well-watered and drought-stressed 'Saji' and well-watered 'Erdyt-89'. Nevertheless, elevation of photosynthesis rate exceeded that of transpiration under these conditions. It resulted in higher instant water use efficiency in drought-stressed plants upon Se treatment in both genotypes.

5 CONCLUSION

Our results indicated that Se application did not change significantly plants biomass under drought conditions but improved some physiological parameters such as photosynthesis, accumulation of osmolytes and water use efficiency but not water relation parameters.

Higher drought sensitivity in 'Erdyt-89' could be attributed to damaged photosynthetic apparatus, failure to limit transpirational water loss and less-efficient accumulation of organic osmolytes under drought conditions compared with 'Saji'. However, two genotypes did not differ in the Se-mediated amelioration of drought stress.

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