COBISS Code 1.01

DOI: 10.14720/aas.2015.105.2.01
Agrovoc descriptors: drought stress, drought, photosynthesis, slenium, water supply, wheats, organic compounds, pigments, field experimentation, protected cultivation

Agris category code: f06,f60

Influence of selenium in drought-stressed wheat plants under greenhouse and field conditions

Roghieh HAJIBOLAND[1], Noushin SADEGHZADEH[2], Nashmin EBRAHIMI[2], Behzad SADEGHZADEH[3] and Seyed Abolgasem MOHAMMADI[4]

Received February 08, 2015; accepted May 19, 2015.
Delo je prispelo 08. februarja 2015, sprejeto 19. maja 2015.

ABSTRACT

Effects of selenium (Na_2SeO_4) was studied in two wheat genotypes under well-watered and drought conditions in greenhouse (15 µg Se L^{-1}) and field (20-60 60 g ha^{-1}) experiments. Application of Se improved dry matter and grain yield under both well-watered and drought conditions. Se increased leaf concentration of pigments and photosynthesis rate under both well-watered and drought conditions. Our results indicated that Se alleviates drought stress via increased photosynthesis rate, protection of leaf photochemical events, accumulation of organic osmolytes and improvement of water use efficiency. Under well-watered condition, Se-mediated growth improvement was associated with higher photosynthesis rate and water use efficiency, greater root length and diameter, and higher leaf water content.

Key words: Drought, Organic osmolytes, Photosynthesis rate, Selenium, Water relations, Wheat

IZVLEČEK

VPLIV SELENA NA PŠENICO V SUŠNEM STRESU V RASTLINJAKU IN NA POLJU

Raziskovan je bil vpliv selena (Na_2SeO_4) na rastline dveh genotipov navadne pšenice, v sušnih razmerah oziroma pri dobri oskrbi z vodo, v rastlinjaku (15 µg Se L^{-1}) in na polju (20-60 60 g ha^{-1}). Dodatek Se je povečal sušino rastlin in pridelek zrnja pri obeh načinih oskrbe z vodo. Se je vplival na povečano koncentracijo pigmentov in na povečanje fotosinteze listov pri obeh oskrbah z vodo. Rezultati kažejo, da dodatek Se omili vpliv sušnega stresa s povečanjem fotosinteze, zaščito lista s fotokemičnimi procesi, akumulacijo organskih ozmotikov in povečano učinkovitostjo porabe vode. V razmerah dobre oskrbe z vodo je bila povečana rast, omogočena z dostopnostjo selena, povezana z intenzivnejšo fotosintezo in večjo učinkovitostjo uporabo vode, daljšimi in debelejšimi koreninami in večjo vsebnostjo vode.

Ključne besede: suša, organski ozmotiki, fotosinteza, selen, vodna oskrba, pšenica

1 INTRODUCTION

Plants often encounter unfavorable conditions, which interrupts their growth and productivity. Among the various abiotic stresses, drought is the major factor that limits crop productivity worldwide (Tardieu et al., 2014). Inadequate water availability during the life cycle of a crop species restricts the expression of its full genetic potential. Most of the crops are sensitive to water deficits, particularly during flowering to seed development stages. Even drought-tolerant crops are adversely

[1] Associate Professor of Plant Physiology, Plant Science Department, University of Tabriz, 51666-14779 Tabriz, Iran

[2] M.Sc. of Plant Physiology, Plant Science Department, University of Tabriz, 51666-14779 Tabriz, Iran

[3] Assistant Professor of Crop Molecular Breeding, Dryland Agricultural Research Institute (DARI), Maragheh, P.O. Box 119, Iran

[4] Professor of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Tabriz, 51666-14779 Tabriz, Iran

This paper is a part of the PhD thesis of N.E. under supervision of R.H.

affected by water scarcity at reproductive stage (Mitra, 2001).

One of the initial responses to water deficiency is stomatal closure that depresses in turn photosynthesis and ability of plants for dry matter production (Chaves et al., 2009; Farooq et al., 2009). Drought-induced reduction of photosynthetic activity, however, is linked also to non-stomatal mechanisms, i.e. reduction of enzyme activities (Chaves et al., 2009). Down-regulation of photosynthetic carbon metabolism leads, in turn, to generation of excess excitation energy and formation of reactive oxygen species that induce damages to photosystems (Hajiboland, 2014). The relative contribution of stomatal and non-stomatal limitations of photosynthesis depends on the severity of water stress and plants susceptibility to desiccation (Chaves et al., 2009).

Plants respond and adapt to water deficit by the accumulation of osmolytes and proteins specifically involved in stress tolerance (Krasensky and Jonak, 2012). These molecules are accumulated in plant cells in response to drought stress and act as osmotic balancing agents. In addition of roles in osmotic homeostasis, these organic solutes are free radical scavengers and protect cell structures and membranes against desiccation damages (Krasensky and Jonak, 2012). Synthesis of osmoprotectants, osmolytes or compatible solutes including amino acids particularly proline and soluble carbohydrates is one of the mechanisms for adaptation to water deficit (Verbruggen and Hermans, 2008). Stress tolerance are controlled also by developmental and morphological traits such as root thickness, the ability of roots to penetrate compacted soil layers, and root depth and mass (Valliyodan and Nguyen, 2006).

To improve production efficiency of crop plants under drought conditions, development of more tolerant genotypes using breeding strategies are necessary (Mitra, 2001; Valliyodan and Nguyen, 2006). Alternatively, exogenous application of various growth regulators (jasmonate and salicylate) and some osmoprotectants (e.g. glycine betaine) has proven worldwide in inducing drought tolerance at various growth stages (Farooq et al., 2009). Among mineral compounds, application of silicon (Liang et al., 2007) and selenium (Se) (Feng et al., 2013) attracted much more attention.

Influence of low concentrations of Se in the amelioration of various abiotic stresses such as salt, UV radiation and drought stresses has been reported for various plant species (Hajiboland, 2012; Feng et al., 2013). Mechanisms for alleviating effect of Se on drought have mainly focused on the Se-mediated activation of antioxidative defense (Wang, 2011; Hasanuzzaman and Fujita, 2011; Hajiboland, 2012; Feng et al., 2013). However, Se-mediated changes in water relation parameters, accumulation of osmoticums and water uptake capacity under drought remain largely unknown and the obtained results are contradictory. Se application was reported to increase the accumulation of proline (Yao et al., 2009) while did not affect water uptake capacity (Habibi, 2013) and did not influence plant biomass under drought (Yao et al., 2009; Habibi, 2013).

Wheat (*Triticum aestivum* L.) is one of the most important staple food crops and its productivity is adversely affected by drought particularly in arid and semi arid regions of the world. It has been estimated that about 50 % of the 237 million ha area in the world under wheat cultivation is affected by periodic drought (Ashraf, 2010). Effect of Se on the mitigation of drought stress in wheat plants has not been studied under field conditions. On the other hand, considering Se as a necessary element for animals and humans, fortification of grains with Se may contribute to an increase in Se intake for humans particularly in the countries where soil Se is low (Lyons et al., 2003).

This work was aimed at studying the effect of Se application on the amelioration of drought stress in wheat genotypes. Some physiological parameters such as gas exchange, water relations and osmolyte accumulation were studied in this crop species in the pot and field experiments in order to compare Se effect as related to growth conditions and developmental stages.

2 MATERIALS AND METHODS

2.1 Plants materials

Two common winter wheat (*Triticum aestivum* L.) genotypes 'Homa' (drought-tolerant) and 'Sara-BW-F6-06-85-86-29-1' (drought-sensitive) were used in both pot experiment and field study. Seeds were provided by Dryland Agricultural Research Institute (DARI) (Maragheh, Iran).

2.2 Pot experiment

Seeds were surface-sterilized and germinated on perlite. Five-days-old seedlings were transferred into 1.5 L pots (40 plants per pot) filled with acid-washed perilte irrigated with 200 ml week^{-1} of 50 % wheat nutrient solution (Hajiboland et al., 2003). Se and irrigation treatments were started 9 days after transplanting, treatments were assigned randomly to the pots. Se treatments at two levels including without and with (15 µg L^{-1}) Se (as Na_2SeO_4) were applied gradually during 4 weeks. Simultaneously, irrigation treatments included well-watered (irrigation at field capacity) and drought stress (irrigation at 20 % FC) were started by omitting watering from drought treatments. One week after withholding watering, pots reached the 20 % 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^{-2}s^{-1} provided by natural light supplemented with fluorescent lamps.

Eight weeks after starting Se treatments (7 weeks after reaching the respective FC, 10 weeks after sowing) plants were harvested. Shoot and roots were separated, roots were washed with distilled water and blotted dry on filter paper and their fresh weight (FW) were determined. Plants dry weight (DW) was determined after drying in 70°C for 48 h. Subsamples from leaves and roots were taken for biochemical analyses before drying. Change in the root morphology in Se-treated plants was visually observed at harvest. For its quantification, the root system of each pot was spread out in a tray filled with distilled water. Thereafter, root length was determined according to the line intersect method (Tennant, 1975) and the root diameter was determined using a micrometer in 50 randomly-selected parts of root system of each replicate pot and the average of obtained data was calculated.

2.3 Field experiment

Field experiment was conducted during the 2012-2013 growing season at the Research Station of Faculty of Agriculture, University of Tabriz. At the beginning of the season (fall of 2012), the experimental area was prepared and soil samples were taken at a depth of 30 cm and analyzed for main soil properties. Soil properties before planting were 76 % sand, 18 % silt, and 6 % clay; 2.1 % organic matter; pH 8.7, EC 33.3 (soil:water, 1:1); 5.0 NO_3-N, 36 P and 480 K in mg kg^{-1} soil. Plot dimensions were 1.5 m×3.0 m containing 5 rows. Seeds of two genotypes were planted by hand in rows and covered with soil. Approximately 60 seeds per row and 300 seeds per plot were planted on 20 October 2012. Weeds were controlled by hand as required. Nitrogen was added at a rate of 60 kg N ha^{-1} as urea in 08 April 2013.

Control and drought-exposed plots were grown under rainfed conditions and supplemental irrigation applied for three times: one day and two weeks after planting and on 08 April 2013. Thereafter, drought was imposed by water withholding while control plots were irrigated weekly up to two weeks before harvest. Soil humidity (%) was estimated in samples collected weekly from drought-imposed plots at 10 cm simultaneous with irrigation of control plots. Soil relative humidity was 12.79±1.2 at the start of withholding irrigation and decreased to 3.52±0.21 at two weeks before harvest without significant difference among plots.

Selenium (as Na_2SeO_4) was sprayed on the leaves at the concentrations of 1 and 3 µg L^{-1} with the final amounts of 20 µg ha^{-1} (Se_1) and 60 µg ha^{-1} (Se_2) respectively, in the mornings before sunrise. Control plots (–Se) were sprayed with distilled water. The first foliar Se treatment was applied 6 weeks after planting. In spring, four subsequent Se

treatments each with 3 weeks interval starting on 09 April 2013 were applied.

At maturity (15 July 2013), plants of each experimental plot were harvested. The harvested material was sun-dried, threshed manually, and weighed for total biomass, grain yield and elemental analyses. Yield components (heads per plant, grains per head, and grain weight) were measured on ten plants that were sampled randomly from the two middle rows of each plot at maturity and added to the total. For determination of K, Ca and P, oven dried leaf samples were ashed in a muffle furnace at 550ºC for 8 h, resolved in HCl, and made up to volume by distilled water. Concentrations of K and Ca were determined by flame photometry (PFP7, Jenway, UK) and P by a spectrophotometer (Specord 200, Analytical *Jena,* Germany) (Jaiswal, 2004).

2.4 Measurement of chlorophyll fluorescence and gas exchange parameters

Chlorophyll (Chl) fluorescence parameters were measured in the pot experiment at harvest using a portable fluorometer (OSF1, ADC Bioscientific Ltd., UK). Leaves were acclimated to dark for 30 min using leaf clips before taking the measurements for dark-adapted leaves. Maximum quantum yield of PSII (F_v/F_m) was calculated using initial (F_0), maximum (F_m) and variable ($F_v=F_m–F_0$) fluorescence parameters. Calculations for light-adapted leaves were undertaken using initial (F_t), steady-state (F_s), maximum (F'_m), variable ($F'_v=F'_m–F_t$), ΦPSII ($F'_m–Ft/F'_m$) and F'_0 [$F'_0=F_0/(F_v/F_m)+(F_0/F'_m)$] fluorescence for excitation capture efficiency of open PSII (F'_v/F'_m), photochemical quenching (qP) [$(F'_m–F_s)/(F'_m–F'_0)$], non-photochemical quenching (qN) [$1–(F'_m–F'_0)/(F_m–F_0)$] ΦPSII ($F'_m–Ft/F'_m$) and electron transport rate (ETR) [ΦPSII×PFD×0.84×0.5] (Krall and Edwards, 1992).

Gas exchange parameters were measured with a calibrated portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK) between 10:00 and 13:00. Measurements in the field experiment were undertaken one day after the last Se application (28 May 2013) in the young and flag leaves. In the greenhouse experiment, gas exchange parameters were measured at three time intervals: 4, 6 and 8 weeks after starting treatments. The measurements were conducted with photosynthetically active radiation (PAR) intensity at the leaf surface of 300-400 μmol m^{-2} s^{-1} in the pot experiment and 800-900 μmol m^{-2} s^{-1} in the field experiment. The net photosynthetic rate by unit of leaf area (A, μmol CO_2 m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}) and the stomatal conductance to water vapor (g_s, mol m^{-2} s^{-1}) were calculated using the values of CO_2 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 $mmol^{-1}$).

2.5 Determination of osmotic potential and relative water content

Osmotic potential was determined in the leaf and root samples using an osmometer (Heman Roebling Messtechnik, Germany). Relative water content (RWC%) was measured in the leaves 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.

2.6 Biochemical determinations

Leaf concentration of Chl a, b and carotenoids (Car) were determined according to Lichtenthaler and Wellburn (1985). 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 $AlCl_3$-metanol (2 %, w/v) as indicator at 510 nm and quercetin (Sigma) as standard (Grayer, 1989).

For determination of non-structural carbohydrates, samples were homogenized in 100 mM phosphate buffer (pH 7.5) at 4 ^oC, 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 Willism, 1954). 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).

2.7 Experimental design and statistical analyses

Pot experiment was undertaken in randomized block design with four replications as four independent pots. Field experiment was arranged in a split-plot-factorial design with four replicates. Watering treatments were the main-plots with Se treatments (–Se, Se_1 and Se_2) and genotypes combinations as sub-plots. Differences between the means were detected according to Tukey's test (p<0.05) using Sigma Stat 2.03 software.

3 RESULTS

Plants fresh and dry matter production was adversely affected by drought under greenhouse conditions (Table 1). Reduction of shoot and root dry weight was 50 % and 29 % in drought tolerant variety 'Homa' and 68 % and 17 % in drought sensitive line 'Sara', respectively. Root length and diameter were also lower in drought-stressed plants. Se application improved all the growth parameters in control plants of both genotypes. In drought-stressed plants, however, effect of Se was significant only for shoot DW and root diameter in 'Homa'. Contrastingly, Se-treated plants tended to have slightly lower root length under drought conditions in both genotypes (Table 1).

Under field conditions, drought stress declined all yield components. Reduction of whole shoot and straw biomass under drought was 40% and 44% in 'Homa', respectively, whereas it was more in 'Sara' (53 % and 56 %, respectively). For reproductive plant parts, yield depression was 23 % and 39 % for spike and seed in 'Homa' respectively, whereas the corresponding values for 'Sara' were 51 % and 54 % (Table 2). Se treatment increased plants growth parameters mainly under well-watered conditions without significant difference between two levels of applied Se. However, these effects were mainly in tendency, and significant changes were observed only for whole shoot and straw weight in 'Homa' (Table 2).

Table 1: Fresh and dry weight (mg plant^{-1}) of shoot and root, root length (mm plant^{-1}) and diameter (mm) in two wheat genotypes grown under control (100 % FC) and drought (20 % FC) conditions in the absence (–Se) or presence (+Se) of Se (15 µg Se L^{-1} as Na_2SeO_4) for 10 weeks in greenhouse.

Treatments		Shoot FW	Shoot DW	Root FW	Root DW	Root length	Root diameter
		'Homa'					
Control	–Se	539±19 ^{b}	119±7 ^{b}	299±36 ^{b}	52±6 ^{ab}	6.07±0.58 ^{b}	0.21±0.009 ^{b}
	+Se	592±28 ^{a}	132±6 ^{a}	346±18 ^{a}	60±4 ^{a}	7.50±0.72 ^{a}	0.30±0.003 ^{a}
Drought	–Se	155±10 ^{c}	60±5 ^{d}	89±12 ^{c}	37±9 ^{c}	2.65±0.57 ^{c}	0.13±0.006 ^{c}
	+Se	163±22 ^{c}	73±3 ^{c}	93±11 ^{c}	39±8 ^{bc}	2.57±0.21 ^{c}	0.21±0.005 ^{b}
		'Sara'					
Control	–Se	566±53 ^{b}	151±15 ^{a}	322±47 ^{b}	69±8 ^{ab}	7.49±0.42 ^{b}	0.18±0.005 ^{c}
	+Se	663±42 ^{a}	168±13 ^{a}	456±14 ^{a}	81±9 ^{a}	7.79±0.34 ^{a}	0.33±0.006 ^{a}
Drought	–Se	126±15 ^{c}	49±12 ^{b}	123±25 ^{c}	57±5 ^{b}	2.69±0.39 ^{c}	0.14±0.002 ^{d}
	+Se	153±26 ^{c}	60±8 ^{b}	136±24 ^{c}	63±5 ^{b}	2.10±0.28 ^{c}	0.28±0.001 ^{b}

Data of each column within each genotype indicated by the same letter are not statistically different (P≤0.05)

Table 2: Various growth parameters including yield of whole shoot, straw, spike and seed (g plant^{-1}) and weight of 1000 seeds (g) in two wheat genotypes grown under control (well-watered) and drought stress conditions without (–Se) or with two levels of foliar-applied Se (Se_1: 20 g ha^{-1} and Se_2: 60 g ha^{-1}) (as Na_2SeO_4) under field conditions.

Treatments		Whole shoot	Straw	Spike	Seed	1000 seeds
		'Homa'				
Control	–Se	19.7±1.6 ^{c}	8.9±1.0 ^{b}	1.07±0.07 ^{a}	7.62±0.72 ^{a}	48.08±3.97 ^{a}
	Se_1	21.5±1.7 ^{ab}	9.9±0.4 ^{ab}	1.15±0.13 ^{a}	8.28±0.68 ^{a}	50.38±4.68 ^{a}
	Se_2	23.7±1.9 ^{a}	12.8±1.2 ^{a}	1.09±0.16 ^{a}	8.08±0.75 ^{a}	44.71±3.82 ^{a}
Drought	–Se	11.8±1.1 ^{d}	5.0±1.0 ^{c}	0.68±0.11 ^{b}	4.63±0.39 ^{b}	35.97±2.29 ^{b}
	Se_1	12.9±0.7 ^{d}	5.4±0.4 ^{c}	0.74±0.04 ^{b}	5.14±0.25 ^{b}	36.37±2.27 ^{b}
	Se_2	13.0±1.2 ^{d}	5.5±1.1 ^{c}	0.75±0.21 ^{b}	4.92±2.10 ^{b}	37.68±3.31 ^{b}
		'Sara'				
Control	–Se	22.8±2.7 ^{a}	8.4±1.2 ^{ab}	1.44±0.16 ^{a}	10.68±0.61 ^{a}	37.57±1.94 ^{a}
	Se_1	25.7±2.4 ^{a}	10.4±2.3 ^{a}	1.53±0.13 ^{a}	11.20±0.97 ^{a}	38.93±2.42 ^{a}
	Se_2	23.2±2.8 ^{a}	11.7±3.9 ^{a}	1.14±0.18 ^{a}	9.70±0.94 ^{a}	38.06±2.21 ^{a}
Drought	–Se	10.7±1.8 ^{b}	3.7±1.1 ^{c}	0.70±0.11 ^{a}	4.89±0.71 ^{b}	27.41±3.18 ^{b}
	Se_1	13.2±1.5 ^{b}	5.4±0.7 ^{bc}	0.77±0.08 ^{a}	5.16±0.56 ^{b}	28.67±3.40 ^{b}
	Se_2	13.2±1.3 ^{b}	5.5±1.0 ^{bc}	0.77±0.13 ^{a}	4.76±0.96 ^{b}	27.30±1.38 ^{b}

Data of each column within each genotype indicated by the same letter are not statistically different ($P \leq 0.05$)

In general, leaf Chl a, b and Car concentrations were higher in drought-stressed plants being significant for Chl a and b in 'Homa'. In contrast, concentrations of anthocyanins were declined by drought treatment in both genotypes and that of flavonoids in 'Homa' (Table 3). Se treatment increased consistently concentrations of all leaf pigments. Compared with –Se treatment, the effect of Se application was much more pronouncedly observed for leaf anthocyanins under both well-watered and drought conditions being about 2.2 and 3.5 folds higher in 'Homa' and 'Sara', respectively. Se affected also significantly Chl a, b in 'Homa' and flavonoids in 'Sara' (Table 3).

Leaf Chl fluorescence parameters were affected by drought condition particularly in 'Homa' (Table 4). Maximum (F_v/F_m) and actual (F'_v/F'_m) efficiency of PSII and electron transport rate (*ETR*) were significantly lowered by drought stress in 'Homa'. Se treatment increased significantly F_v/F_m and *ETR* as well as non-photochemical quenching (*qN*) in 'Homa' under drought stress (Table 4).

Table 3: Concentration of leaf pigments (mg g^{-1} FW) in two wheat genotypes grown under control (100 % FC) and drought (20 % FC) conditions in the absence (–Se) or presence (+Se) of Se (15 µg Se L^{-1} as Na_2SeO_4) for 10 weeks in greenhouse.

Treatments		Chl *a*	Chl *b*	Car	Anthocyanins	Flavonoids
		'Homa'				
Control	–Se	1.66±0.32 ^{b}	0.81±0.05 ^{b}	0.50±0.08 ^{a}	7.98±1.46 ^{ab}	5.65±0.44 ^{a}
	+Se	2.29±0.30 ^{a}	0.94±0.05 ^{a}	0.52±0.07 ^{a}	9.78±2.75 ^{a}	5.87±0.34 ^{a}
Drought	–Se	2.31±0.11 ^{a}	0.98±0.07 ^{a}	0.57±0.03 ^{a}	2.30±1.30 ^{c}	3.78±0.08 ^{c}
	+Se	2.53±0.18 ^{a}	1.05±0.06 ^{a}	0.60±0.05 ^{a}	5.28±1.28 ^{bc}	4.88±0.24 ^{b}
		'Sara'				
Control	–Se	2.06±0.30 ^{b}	0.88±0.05 ^{a}	0.46±0.08 ^{a}	9.35±2.83 ^{bc}	3.10±0.14 ^{c}
	+Se	2.13±0.19 ^{ab}	0.93±0.02 ^{a}	0.49±0.04 ^{a}	18.0±2.53 ^{a}	4.51±0.24 ^{b}
Drought	–Se	2.25±0.21 ^{ab}	0.90±0.07 ^{a}	0.53±0.06 ^{a}	4.01±1.35 ^{c}	4.21±0.33 ^{b}
	+Se	2.55±0.17 ^{a}	0.98±0.08 ^{a}	0.57±0.05 ^{a}	13.89±3.74 ^{ab}	5.50±0.07 ^{a}

Data of each column within each genotype indicated by the same letter are not statistically different ($P \leq 0.05$)

Table 4: Chlorophyll fluorescence parameters: F_v/F_m (maximum quantum efficiency of PSII), F'_v/F'_m (excitation energy capture of PSII), *qP* (photochemical quenching), *qN* (non-photochemical quenching) and *ETR* (electron transport rate) in two wheat genotypes grown under control (100 % FC) and drought (20 % FC) conditions in the absence (–Se) or presence (+Se) of Se (15 µg Se L^{-1} as Na_2SeO_4) for 10 weeks in greenhouse.

Treatments		F_v/F_m	F'_v/F'_m	*qP*	*qN*	*ETR*
		'Homa'				
Control	–Se	0.86±0.00 [a]	0.75±0.00 [a]	0.99±0.02 [a]	0.07±0.04 [b]	124±3 [a]
	+Se	0.86±0.00 [a]	0.76±0.01 [a]	0.98±0.03 [a]	0.09±0.02 [b]	125±2 [a]
Drought	–Se	0.84±0.01 [b]	0.72±0.02 [b]	0.99±0.01 [a]	0.12±0.08 [b]	117±3 [b]
	+Se	0.86±0.01 [a]	0.72±0.01 [b]	0.98±0.01 [a]	0.25±0.05 [a]	120±3 [a]
		'Sara'				
Control	–Se	0.86±0.01 [a]	0.73±0.01 [a]	0.98±0.02 [a]	0.15±0.03 [a]	122±2 [a]
	+Se	0.87±0.01 [a]	0.74±0.01 [a]	0.95±0.03 [a]	0.20±0.02 [a]	118±5 [ab]
Drought	–Se	0.85±0.02 [a]	0.72±0.01 [a]	0.95±0.02 [a]	0.16±0.03 [a]	117±2 [ab]
	+Se	0.85±0.02 [a]	0.72±0.01 [a]	0.98±0.01 [a]	0.21±0.04 [a]	115±2 [b]

Data of each column within each genotype indicated by the same letter are not statistically different ($P \leq 0.05$)

Four weeks after starting treatments, a significant reduction of stomatal conductance was observed only in 'Homa'. Expectedly, transpiration rate was also lowered by drought stress but these changes were not statistically significant. In contrast, reduction of net photosynthesis rate by drought stress was significant in both genotypes (Table 5).

Se treatment in well-watered plants increased stomatal conductance, transpiration and photosynthesis rates in 'Homa' while decreased the latter parameter in 'Sara'. In drought-stressed plants Se application caused reduction of net assimilation rate in both genotypes (Table 5). Under long-term (6 and 8 weeks) drought stress, however, stomatal conductance and transpiration rates were significantly lowered in both genotypes. Se application increased all gas exchange parameters slightly or significantly in both genotypes. Eight weeks after starting treatments, leaf photosynthesis rate was increased by Se up to 23 % and 120 % in drought-stressed 'Homa' and 'Sara', respectively (Table 5). Gas exchange parameters under field conditions responded in the same way as observed in the pot experiment (Table 6).

Stomatal conductance was significantly lowered by drought stress in both young and flag leaves. A significant reduction of transpiration rate under drought stress, however, was observed only in the young and flag leaves of 'Sara'. Se-treated plants had higher stomatal opening and transpiration rate particularly in the flag leaves and two application levels of Se did not differ in this regard. Net assimilation rate was affected by both drought and Se treatments more pronouncedly than other two parameters. Both young and flag leaves had lower photosynthesis rate under drought irrespective to the level of Se treatments. In turn, Se application resulted in significantly higher photosynthesis under both watering treatments and in both genotypes. In contrast to stomatal conductance and transpiration rate, effect of higher Se concentration (60 g ha^{-1}) on the elevation of photosynthesis was greater than that of lower Se concentration (20 g ha^{-1}) (Table 6). Furthermore, the extent of Se effect on the increases in leaf photosynthesis of drought-stressed plants was higher under field conditions and reached up to 162 % and 179 % in the young leaves and 191 % and 202 % in the flag leaves of 'Homa' and 'Sara', respectively.

Table 5: Gas exchange parameters including net assimilation rate (A, μmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}) and stomatal conductance (g_s, mol m^{-2} s^{-1}) in two wheat genotypes grown under control (100 % FC) and drought (20 % FC) conditions in the absence (–Se) or presence (+Se) of Se (15 μg Se L^{-1} as Na_2SeO_4) at three measurement intervals in greenhouse.

Treatments		*A*	*E*	g_s	*A*	*E*	g_s
		'Homa'			'Sara'		
		4 weeks after starting treatments					
Control	–Se	13.71±0.91 [b]	2.08±0.70 [b]	0.50±0.09 [a]	15.09±0.02 [a]	1.42±0.09 [b]	0.23±0.16 [b]
	+Se	15.80±0.45 [a]	4.70±0.26 [a]	0.58±0.13 [a]	12.81±0.30 [b]	2.58±0.14 [a]	0.70±0.18 [a]
Drought	–Se	11.96±0.32 [c]	1.42±0.27 [bc]	0.26±0.05 [b]	11.06±0.33 [c]	1.43±0.63 [b]	0.14±0.05 [b]
	+Se	7.79±0.18 [bc]	0.81±0.22 [c]	0.10±0.04 [b]	9.08±0.56 [d]	1.16±0.40 [b]	0.21±0.08 [b]
		8 weeks after starting treatments					
Control	–Se	15.69±0.18 [b]	1.58±0.11 [a]	1.33±0.05 [a]	10.33±0.30 [c]	1.19±0.11 [b]	1.77±0.24 [a]
	+Se	18.04±0.45 [a]	1.60±0.15 [a]	1.41±0.17 [a]	12.52±0.24 [a]	1.57±0.06 [a]	1.91±0.12 [a]
Drought	–Se	8.61±0.49 [d]	0.93±0.05 [b]	0.90±0.21 [b]	9.91±0.48 [c]	0.86±0.02 [c]	0.38±0.09 [b]
	+Se	10.75±0.69 [c]	0.98±0.05 [ab]	1.24±0.19 [ab]	11.24±0.52 [b]	0.91±0.04 [c]	0.57±0.08 [b]
		10 weeks after starting treatments					
Control	–Se	16.70±0.54 [b]	1.52±0.34 [ab]	1.49±0.14 [ab]	8.85±0.13 [b]	1.43±0.36 [ab]	1.94±0.68 [a]
	+Se	19.02±0.36 [a]	1.72±0.48 [a]	2.06±0.18 [a]	13.01±0.45 [a]	1.99±0.33 [a]	2.44±0.56 [b]
Drought	–Se	5.86±0.13 [d]	0.92±0.04 [b]	0.67±0.14 [b]	2.14±0.20 [d]	0.78±0.11 [c]	0.10±0.03 [c]
	+Se	7.20±0.44 [c]	0.97±0.06 [b]	0.91±0.17 [b]	4.71±0.44 [c]	0.93±0.19 [bc]	0.11±0.06 [bc]

Data of each column within each measurement intervals indicated by the same letter are not statistically different ($P \leq 0.05$)

Table 6: Gas exchange parameters including net assimilation rate (A, μmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}) and stomatal conductance (g_s, mol m^{-2} s^{-1}) in the young and flag leaves in two wheat genotypes grown under control (well-watered) and drought stress conditions without (–Se) or with two levels of foliar-applied Se (Se_1: 20 g ha^{-1} and Se_2: 60 g ha^{-1}) (as Na_2SeO_4) under field conditions.

Treatments		*A*	*E*	g_s	*A*	*E*	g_s
		'Homa'			'Sara'		
		Young leaf					
Control	–Se	8.43±0.39 [c]	1.55±0.90 [ab]	0.35±0.04 [a]	8.48±0.36 [c]	1.21±0.17 [ab]	0.42±0.15 [ab]
	Se_1	11.95±0.38 [b]	1.86±0.37 [a]	0.55±0.17 [a]	13.14±0.72 [b]	1.51±0.62 [a]	0.61±0.10 [a]
	Se_2	15.27±0.82 [a]	1.51±0.66 [ab]	0.42±0.15 [a]	15.98±0.66 [a]	1.04±0.12 [abc]	0.43±0.13 [ab]
Drought	–Se	1.62±0.32 [f]	0.39±0.07 [b]	0.07±0.01 [b]	1.88±0.06 [e]	0.44±0.10 [c]	0.23±0.15 [b]
	Se_1	2.61±0.17 [e]	0.81±0.41 [ab]	0.09±0.04 [b]	2.97±0.38 [e]	0.53±0.09 [c]	0.30±0.19 [ab]
	Se_2	4.25±0.25 [d]	0.84±0.48 [ab]	0.14±0.02 [b]	5.24±0.80 [d]	0.68±0.09 [bc]	0.22±0.09 [b]
		Flag leaf					
Control	–Se	9.75±0.19 [c]	0.71±0.06 [b]	0.19±0.01 [bc]	9.95±0.17 [c]	0.65±0.09 [b]	0.24±0.05 [b]
	Se_1	12.0±0.12 [b]	1.08±0.24 [ab]	0.31±0.12 [ab]	15.6±0.21 [b]	0.94±0.08 [a]	0.49±0.16 [a]
	Se_2	14.9±0.54 [a]	1.15±0.25 [a]	0.34±0.08 [a]	17.3±0.11 [a]	0.91±0.09 [a]	0.48±0.14 [a]
Drought	–Se	1.51±0.17 [f]	0.77±0.20 [b]	0.06±0.03 [c]	1.75±0.15 [f]	0.26±0.00 [c]	0.06±0.01 [b]
	Se_1	3.55±0.22 [e]	1.13±0.39 [ab]	0.09±0.02 [c]	4.40±0.05 [e]	0.54±0.08 [b]	0.14±0.03 [b]
	Se_2	4.41±0.31 [d]	1.56±0.11 [a]	0.12±0.02 [c]	5.28±0.42 [d]	0.70±0.07 [b]	0.24±0.05 [b]

Data of each column within each type of leaf indicated by the same letter are not statistically different ($P \leq 0.05$)

Soluble protein concentration in the shoot was decreased slightly by drought treatment in 'Homa' while increased significantly in 'Sara' (Fig. 1A). In the roots, both genotypes had higher soluble proteins under drought conditions. Se treatment increased consistently soluble protein concentrations in the shoot and roots of both genotypes being significant in 'Homa' under drought and in 'Sara' under well-watered conditions (Fig. 1A).

Similar with soluble proteins, shoot concentration of free amino acids decreased upon drought treatment in 'Homa' while increased significantly in 'Sara' (Fig. 1B). In the roots of both genotypes higher free α-amino acids concentration was found in drought-stressed plants compared with well-watered ones. Effect of Se treatment depended on the plant organ, it resulted in slightly lower free amino acids concentration in the shoot while the opposite was observed in the roots that was significant in drought-stressed plants (Fig. 1B).

Expectedly, concentration of free proline was higher in the plants exposed to drought stress (Fig. 1C). This effect was more pronouncedly observed in 'Homa' compared with 'Sara' in both shoot and roots. Se effect on the shoot proline concentration of drought-stressed plants, however, differed between two genotypes. It decreased proline concentration of drought-stressed plants in 'Homa' whereas increased it in 'Sara'. In the roots, in contrast, both genotypes responded similarly to Se application as slightly or significantly lower proline concentration (Fig. 1C).

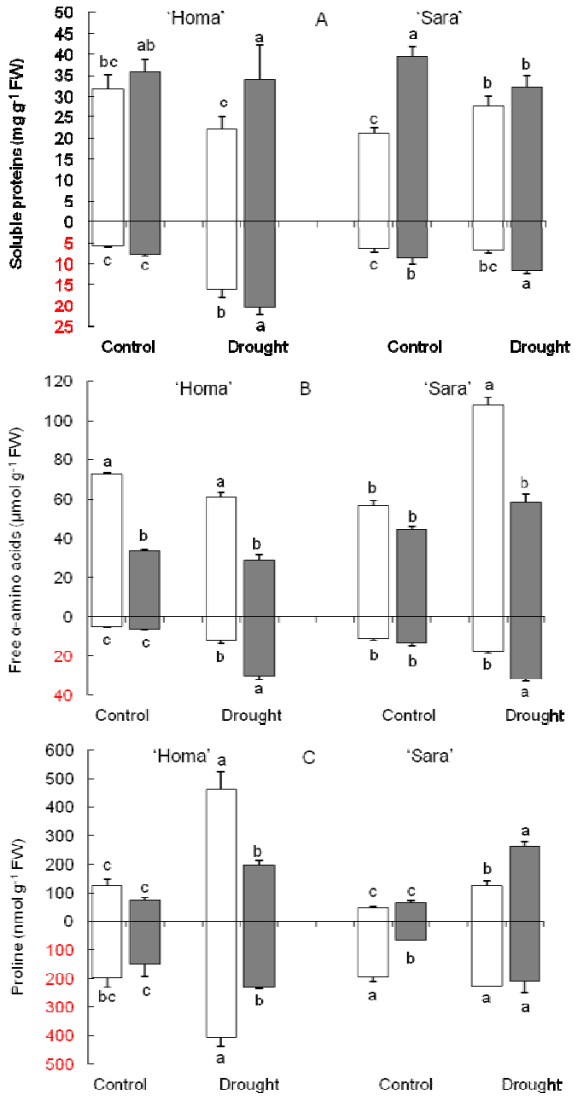

Figure 1: Concentration of soluble proteins (A) (mg g^{-1} FW), total free α-amino acids (B) (μmol g^{-1} FW) and proline (C) (nmol g^{-1} FW) in the leaves (above of the horizontal lines) and roots (below of the horizontal lines) of two wheat genotypes grown under control (100 % FC) and drought (20 % FC) conditions in the absence (–Se, open bars) or presence (+Se, closed bars) of Se (15 μg Se L^{-1} as Na_2SeO_4) for 10 weeks in greenhouse. Data of each column within each genotype indicated by the same letter are not statistically different ($P \leq 0.05$)

Concentration of soluble carbohydrates increased consistently by both drought stress and Se treatments in both genotypes in the shoot and roots (Fig. 2A). This led to the highest amount of soluble sugars in drought-stressed plants supplemented with Se. Root's concentration of starch showed the same pattern of changes in the soluble sugars concentration in response to the treatments. In the shoot, however, starch concentration was not affected by drought in both genotypes and increased by Se application only in 'Homa' under well-watered conditions.

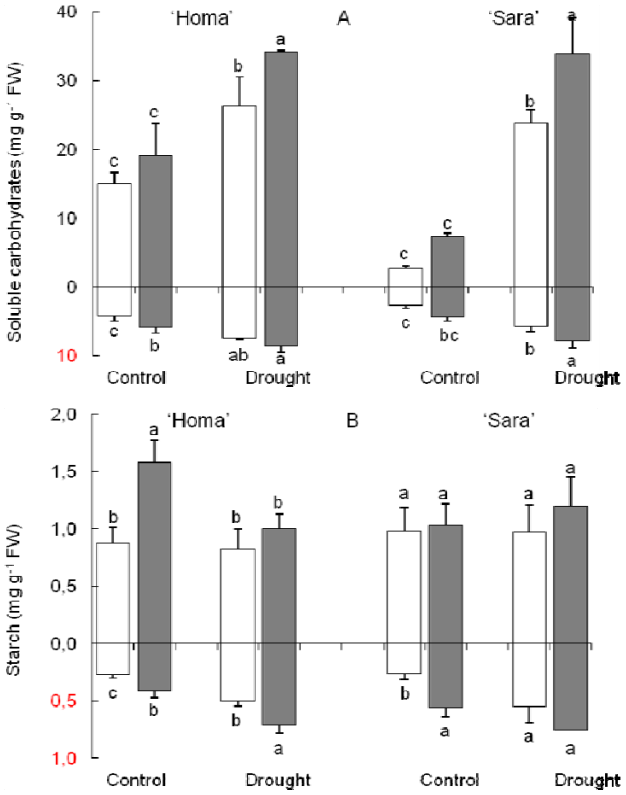

Figure 2: Concentration (mg g^{-1} FW) of soluble sugars (A) and starch (B) in the leaves (above of the horizontal lines) and roots (below of the horizontal lines) of two wheat genotypes grown under control (100 % FC) and drought (20 % FC) conditions in the absence (–Se, open bars) or presence (+Se, closed bars) of Se (15 µg Se L^{-1} as Na_2SeO_4) for 10 weeks in greenhouse. Data of each column within each genotype indicated by the same letter are not statistically different ($P \leq 0.05$).

Expectedly, leaf and root osmotic potentials decreased by drought stress in both genotypes (Fig. 3A). Effect of drought on reduction of roots osmotic potential was more pronounced in 'Homa' than 'Sara'. Se-treated plants had consistently higher leaf osmotic potential that was significant in 'Homa' under drought and in 'Sara' under well-watered conditions. Surprisingly, Se effect on the root osmotic potential was the opposite of that observed for the shoot. A significant effect of Se on declining root osmotic potential was observed in 'Homa' grown under drought conditions (Fig. 3B).

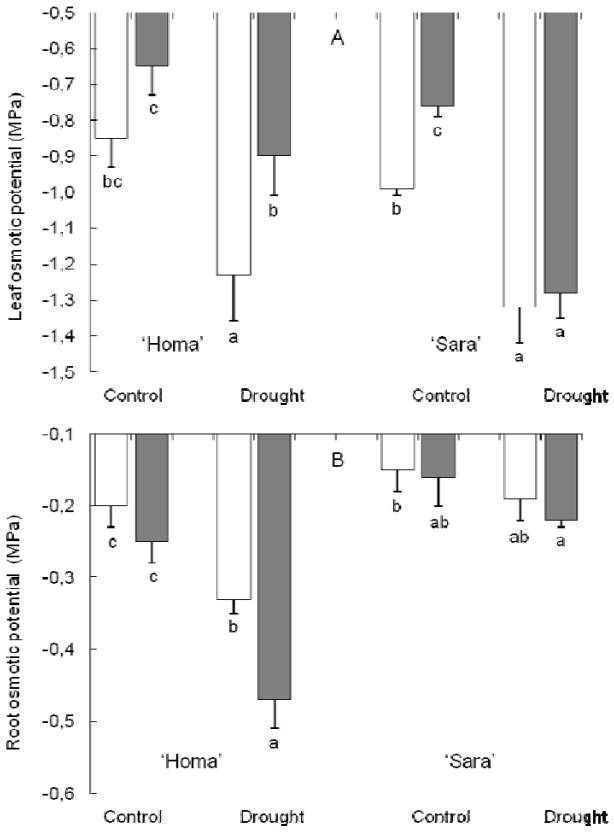

Figure 3: Leaf (A) and root (B) osmotic potentials (MPa) in two wheat genotypes grown under control (100 % FC) and drought (20 % FC) conditions in the absence (–Se, open bars) or presence (+Se, closed bars) of Se (15 µg Se L^{-1} as Na_2SeO_4) for 10 weeks in greenhouse. Data of each column within each genotype indicated by the same letter are not statistically different ($P \leq 0.05$)

Leaf RWC increased significantly by Se application in 'Homa' under well-watered conditions and in 'Sara' under drought stress. Instant *WUE* declined under drought conditions in both genotypes and in greenhouse and field experiments. Se treatment, however, increased this parameter slightly or significantly, under both watering treatments (Table 7).

Leaf concentration of K was lower in drought-stressed plants while that of Ca increased under these conditions in both genotypes. However, effect of drought on K concentration was not significant in 'Sara' (Table 8). Se application did not change K and Ca concentration in the leaves. In contrast to K and Ca, concentration of P in 'Homa' did not respond to drought while it was higher at higher Se application level (60 g ha^{-1}) under both watering regimes. In 'Sara', however, Se did not affect significantly P concentration of leaves while it was slightly lower in drought-stressed plants compared with well-watered ones (Table 8).

Table 7: Relative water content (RWC%) and instant water use efficiency (*WUE*, photosynthesis rate: transpiration rate) in two wheat genotypes grown under control or drought conditions in the absence (–Se) or presence (+Se) of added Se.

Treatments		RWC*	WUE*	WUE**	RWC*	WUE*	WUE**
		'Homa'			'Sara'		
Control	–Se	78.9±1.53 ^{b}	10.67±1.25 ^{ab}	6.49±1.52 ^{b}	84.7±1.35 ^{a}	6.24±0.98 ^{a}	7.07±0.76 ^{b}
	+Se	89.6±1.94 ^{a}	11.11±1.98 ^{a}	11.59±2.67 ^{a}	86.1±1.09 ^{a}	6.61±0.42 ^{a}	15.47±1.77 ^{a}
Drought	–Se	75.1±0.74 ^{c}	6.31±0.98 ^{c}	4.18±0.18 ^{b}	70.9±1.61 ^{c}	2.71±0.81 ^{b}	4.53±1.45 ^{b}
	+Se	77.0±1.92 ^{bc}	7.41±1.82 ^{bc}	6.02±1.21 ^{b}	75.0±1.94 ^{b}	5.11±0.70 ^{a}	7.86±1.78 ^{b}

Data of each column indicated by the same letter are not statistically different ($P \leq 0.05$)
* Measured or calculated for plants grown in greenhouse 10 weeks after starting treatments
** Calculated for young leaves of plants grown in field at Se level of 60 µg ha-1

Table 8: Concentration (mg g^{-1} DW) of K, Ca and P in the leaves of two wheat genotypes grown under control (well-watered) and drought conditions without (–Se) or with two levels of foliar-applied Se (Se_1: 20 g ha^{-1} and Se_2: 60 g ha^{-1}) (as Na_2SeO_4) under field conditions.

Treatments		K	Ca	P	K	Ca	P
		'Homa'			'Sara'		
Control	–Se	165±12 ^{a}	4.82±0.53 ^{b}	0.56±0.24 ^{b}	195±16 ^{a}	5.33±0.98 ^{d}	0.78±0.20 ^{a}
	Se_1	165±13 ^{a}	4.96±0.89 ^{b}	0.58±0.10 ^{b}	181±10 ^{ab}	7.39±0.86 ^{cd}	0.65±0.13 ^{ab}
	Se_2	178±19 ^{a}	4.91±0.52 ^{b}	1.08±0.33 ^{a}	183±12 ^{ab}	5.52±0.80 ^{d}	0.65±0.08 ^{ab}
Drought	–Se	128±18 ^{b}	8.37±1.31 ^{a}	0.35±0.03 ^{b}	171±11 ^{ab}	10.6±1.73 ^{bc}	0.41±0.08 ^{b}
	Se_1	128±15 ^{b}	9.48±1.92 ^{a}	0.37±0.08 ^{b}	163±17 ^{b}	12.8±1.25 ^{b}	0.43±0.11 ^{b}
	Se_2	132±2 ^{b}	7.82±1.76 ^{a}	1.16±0.11 ^{a}	167±4 ^{b}	17.2±2.58 ^{a}	0.49±0.04 ^{b}

Data of each column indicated by the same letter are not statistically different ($P \leq 0.05$)

4 DISCUSSION

4.1 Effect of drought and Se application on plants biomass

Plants biomass was significantly influenced by drought stress under both greenhouse and field conditions. The comparison of two genotypes showed that 'Sara' was more susceptible to drought stress compared with 'Homa' considering vegetative and reproductive stages. It was consistent with the instruction of providing institute on the ranking of drought tolerance in these genotypes. Beside the loss of cell turgor and reduction of cell expansion, the main mechanism reduce crop yield under drought conditions is lowered canopy absorption of photosynthetically active radiation following prolonged stomatal closing (Farooq et al., 2009). Reduction of seed yield and weight under drought, in turn, is related to both source and sink limitations. Apart from source limitation due to reduction of photosynthesis and limited sucrose export to the reproductive sinks, lower capacity of developing seeds to utilize the incoming assimilates and enhanced endogenous abscisic acid concentration are potential factors contributing to reduction of seed yield and weight under drought (Farooq et al., 2009).

Under well-watered conditions, Se application increased vegetative biomass of both genotypes in greenhouse. Effect of Se under field conditions, however, was significant only in 'Homa' and for vegetative but not reproductive growth parameters. Se at 20 g ha^{-1} could be regarded as optimum concentration because higher level (60 g ha^{-1}) did not improve significantly the vegetative growth and reduced slightly reproductive growth parameters. Root diameter was significantly higher in Se-treated plants under well-watered and drought conditions. It has been demonstrated that larger diameter roots would confer drought resistance because they have greater xylem vessel radii and lower axial resistance to water flux, with great penetration ability (Gowda et al., 2011).

Higher P (and partly K) concentration at higher Se application level (60 g ha^{-1}) resulted likely from Se-mediated changes in the root morphology (length and diameter) that improved uptake of nutrients particularly those with higher dependency to spatial availability.

4.2 Effect of drought and Se application on leaf pigments, photochemistry and gas exchange

Leaf concentrations of Chl (significantly) and Car (slightly) was higher in drought-stressed plants obviously because of a concentration effect following higher reduction of leaf weight and area but less destruction of Chl and Car. Reduction of leaf Chl concentration and damages to chloroplasts occur under drought stress when particularly associated with higher light intensity (Hajiboland, 2014). In our greenhouse experiment, drought stress in the absence of higher light intensity, was likely not effective for a high generation rate of reactive oxygen species, chloroplasts damages and Chl destruction.

Nevertheless, anthocyanins and flavonoids concentrtaions rather decreased under water deficiency conditions. It implies that effect of leaf desiccation on the anthocyanins and flavonoids synthesis was much more than that for Chl and Car.

The most prominent effect of Se on leaf pigments was observed for anthocynainns and flavonoids. Accumulation of anthocyanins is believed to protect the cellular structures from oxidative damage (Wahid and Ghazanfar, 2006). Plant tissues containing anthocyanins are usually tolerant to drought (Ichikawa et al., 2001). This protection is related to the superoxide radical scavenging activity of anthocyanins (Ichikawa et al., 2001) and their ability to stabilize the water potential (Chalker-Scott, 2002). The contribution of flavonoids 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 free radicals-scavenging system in plants exposed to severe and prolonged stress conditions (Fini et al., 2011).

In contrast to growth parameters, leaf photochemical parameters were affected adversely by drought in ‘Homa’ but not in ‘Sara’. The preservation of Chl fluorescence parameters in ‘Sara’ under drought conditions indicated that photochemical events conserved their normal activities in this genotype. Reduction of optimal photochemical efficiency of PSII in dark-adapted leaves (F_v/F_m) and excitation capture efficiency of light-adapted leaves (F'_v/F'_m) both indicated occurrence of photoinhibition and damage to PSII in ‘Homa’. Environmental stresses such as drought and salinity reduce the activity of the Calvin cycle directly or indirectly, and result in a decline of $NADP^+$ regeneration, thus, over-reduction of the electron transport chain and photoinhibition and dames to the photosystems (Hajiboland, 2014; Noctor et al., 2014). Amounts of F_v/F_m, F'_v/F'_m and *ETR* under drought conditions was completely returned by Se application to its control levels in ‘Homa’. In addition, higher non-photochemical quenching (*qN*) in drought-stressed plants due to Se application observed in this study implied elevation of capacity for dissipation of excess absorbed energy as heat and thus more protection of photosynthetic apparatus (Müller et al., 2001).

Stomatal conductance was decreased significantly 4 weeks after imposing drought in ‘Homa’ leading to lower transpiration rate in this genotype. This effect, however, was less pronouncedly observed in ‘Sara’ without reduction of transpiration rate. In the two following measurement intervals in greenhouse and field experiment, however, both genotypes showed similar reduction of stomatal conductance and transpirational water loss. Stomatal closure is a fast response of plants to water deficit allowing reduction of the transpiration rate and conservation of relative water content (Zhou et al., 2013). Reduction of stomatal conductance under drought decreased net assimilation rate that was significant at all measurement intervals in the greenhouse experiment and in both leaf types in the field experiment. In addition, reduction of CO_2 availability for photosynthetic dark reactions may result in generation of excess excitation energy under drought conditions and causes damages to photosynthetic apparatus (Hajiboland, 2014) that was also reflected in Chl fluorescence parameters in this work.

Se treatment influenced gas exchange parameters more prominently than photochemical events. In general, stomatal conductance, transpiration rate

and particularly net assimilation rates were higher in Se-treated plants compared with plants without Se under both watering regimes. A significant elevation of net assimilation rate may explain growth improvement by Se not only under drought but also under well-watered conditions. Higher ability for CO_2 fixation increases plants capability for dry matter production under well-watered and for osmolytes accumulation under drought conditions. However, significantly higher photosynthesis rate at 60 g ha^{-1} compared with application of lower Se (20 g ha^{-1}) in well-watered plants was not associated with higher vegetative or reproductive growth parameters. Similarly, an elevated photosynthetic rate up to 3 fold did not result in significantly higher biomass in drought-stressed plants. Lack of a close relationship between photosynthesis rate and plants growth under both watering regimes suggested that there were some limiting factors e.g. low nutrients availability, which prevent maximum response of dry matter production to added Se.

Mechanisms for Se-mediated increase in photosynthesis rate have not been studied in detail. Possible effect of Se on the H^+-pumping and K^+ currents in stomatal cells has not been characterized so far. In addition, non-stomatal mechanisms are also likely involved in the Se effect on photosynthesis. Through proteomic analysis, it was revealed that primary metabolism, photosynthesis and redox homeostasis are the most highly affected biological processes by Se treatments (Wang et al., 2012). Effect of Se on the activation of fructose 1,6 bisphosphatase in alfalfa (Owusu-Sekyere et al., 2013) and a concomitant activation of NO_3^- and CO_2 assimilation rate in wheat (Hajiboland and Sadeghzadeh, 2014) have been previously reported.

4.3 Effect of Se application and drought on the organic osmolytes

Increases in soluble proteins in both leaves and roots of Se-treated plants under both watering regimes that was associated with lower free α-amino acids in the leaves (but not in the roots) may imply depletion of amino acids pool following elevated protein synthesis by Se in the leaves. Lack of such depletion in the roots may be attributed to higher nitrate uptake and assimilation exceeding the requirement for protein synthesis in this organ. Our previous study showed that Se-treated plants have higher nitrate uptake and nitrate reductase activity being much more pronounced in the roots compared with the leaves (Hajiboland and Sadeghzadeh, 2014). Higher concentrations of soluble proteins and free amino acids under drought observed here may confer higher osmotic adjustment capacity and protect cell structures against desiccation (Krasensky and Jonak, 2012).

Proline concentration was expectedly higher in drought-stressed plants whereas Se effect depended on plants organ and genotype. In the roots of both genotypes, Se treatment did not cause accumulation of proline despite lower root osmotic potential. It has been stated that, lower proline accumulation is mainly a reflection of an increased salt resistance in plants, i.e. less injury (Lutts et al., 1999). Here lower proline content of Se-treated plants under drought suggests that they were less strained due to some ameliorating mechanisms. In the leaves genotypic difference was observed in proline accumulation in response to Se. In another study, drought-stressed wheat had higher leaf proline concentration when exposed to Se (Yao et al., 2009).

Desiccation-induced accumulation of soluble sugars observed in this work is a well-known response 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). 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). Se-mediated accumulation of soluble sugars and starch has previously been reported in lettuce (Pennanen et al., 2002) and potato (Turakainen et al., 2004). However, effect of Se application on this parameter under different water supply level and its role in drought tolerance has not been investigated so far. In this work, Se treatment increased concentration of soluble sugars in both leaves and roots of well-watered and drought-stressed plants. It may be primarily attributed to the higher photosynthesis and CO_2 assimilation. This suggestion is confirmed by concomitantly higher starch concentration of Se-treated plants that in turn, excludes the contribution of starch

degradation to the increased concentration of soluble sugars upon Se application.

4.4 Effect of Se application and drought on water relation parameters

Se treatment increased leaf osmotic potentials while decreased it in the roots. Such differential effect of Se on leaf and root osmotic potentials may be resulted from higher root-shoot water transport that could be related in turn, to higher root hydraulic conductivity in Se-treated plants. Root-to-leaf conductance declines during drought and have the greater influence under transpirational conditions compared with soil-to-root component of water pathway (Sperry, 2000). Much of the decline in root-to-shoot hydraulic conductance could be explained by xylem cavitation under drought (Sperry, 2000). Regarding changes in the root morphology and increase in the ratio of thick roots in the wheat plants of this study under Se treatment it could be speculated that hydraulic conductivity of whole root system was higher in Se-treated plants likely because of higher diameter of xylem conduits in the thick roots as was observed in the tick roots of rice (Gowda et al., 2011). To our best knowledge, there is no published work on the changes in water relation parameters as affected by Se in drought-stressed plants. Thus, comparison of our results with other works was not possible. Regardless of mechanism, elevation of leaf osmotic potential upon Se treatment may contribute significantly to the maintenance of biochemical reactions under drought. Lower root osmotic potential, in turn, may allow plants to take up water more efficiently from dry substrate. This parameter together with greater root diameter plays likely important roles for improvement of water uptake capacity in Se-treated plants.

5 REFERENCES

Ashraf, M. 2010 Inducing drought tolerance in plants: Recent advances. Biotechnol. Adv. 28: 169-183, DOI: 10.1016/j.biotechadv.2009.11.005

Bates, L.S., Waldren, R.P., Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39: 205-207, DOI: 10.1007/BF00018060

Chalker-Scott. L. 2002. Do anthocyanins function as osmoregulators in leaf tissues? Adv. Bot. Res. 37: 103-106, DOI: 10.1016/S0065-2296(02)37046-0

Chaves, M.M., Flexas, J., Pinheiro, C. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103: 551-560.DOI: 10.1093/aob/mcn125

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A. 2009. Plant drought stress: effects, mechanisms and management. Agron. Sustain. Dev. 29: 185-212, DOI: 10.1051/agro:2008021

Feng, R., Wei, C., Tu, S. 2013. The roles of selenium in protecting plants against abiotic stresses. Environ. Exp. Bot. 87: 58-68, DOI: 10.1016/j.envexpbot.2012.09.002

Fini, A., Brunetti, C., Di Ferdinando, M., Ferrini, F., Tattini, M. 2011. Stress-induced flavonoid biosynthesis and the antioxidant machinery of plants. Plant Signal. Behav. 6: 709-711, DOI: 10.4161/psb.6.5.15069

Giusti, M.M., Wrolstad, R.E. 2001. Characterization and measurement of anthocyanins by UV–Visible spectroscopy. In: Current Protocols in Food Analytical Chemistry. Wrolstad, R.E., Acree, T.E., An, H., Decker, E.A., Pennere, M.H., Reid, D.S., Schwartz, S.J., Shoemaker, C.F., Sporns, P. (eds.). New York, John Wiley: F1.2.1–F1.2.13, DOI: 10.1002/0471142913.faf0102s00

Gowda, V.R.P., Henry, A., Yamauchi, A., Shashidhar, H.E., Serraj, R. 2011. Root biology and genetic improvement for drought avoidance in rice. Field Crops Res. 122: 1–13, DOI: 10.1016/j.fcr.2011.03.001

Grayer, R.J. 1989. Flavonoids. In: Methods in Plant Biochemistry, Vol. 1, Plant Phenolics. Dey, P.M., Harborne, J.B. (eds.) London, academic Press: 283-323.

Habibi, G. 2013. Effect of drought stress and selenium spraying on photosynthesis and antioxidant activity of spring barley. Acta Agric. Slov. 101: 31-39, DOI: 10.2478/acas-2013-0004

Hajiboland, R. 2012. Effect of micronutrient deficiencies on plant stress responses. In: Abiotic Stress Responses in Plants. Ahmad, P., Prasad, M.N.V. (eds.). New York, Springer: 283-329, DOI: 10.1007/978-1-4614-0634-1_16

Hajiboland, R. 2014. Reactive oxygen species and photosynthesis. In: Oxidative Damage to Plants,

Antioxidant Networks and Signaling. Ahmad, P. (ed.). New York, Springer: 1-63, 10.1016/B978-0-12-799963-0.00001-0

Hajiboland, R., Sadeghzadeh, N. 2014. Effect of selenium supplementation on CO_2 and NO_3^- assimilation under low and adequate N supply in wheat (*Triticum aestivum* L.) plants. Photosynthetica, DOI: 10.1007/s11099-014-0058-1.

Hajiboland, R., Yang, X.E., Römheld, V. 2003. Effects of bicarbonate and high pH on growth of Zn-efficient and Zn-inefficient genotypes of rice, wheat and rye. Plant Soil 250: 349-357, DOI: 10.1023/A:1022862125282

Hasanuzzaman, M., Fujita, M. 2011. Selenium pretreatment upregulates the antioxidant defense and methylglyoxal detoxification system and confers enhanced tolerance to drought stress in rapeseed seedlings. Biol. Trace Element Res. 143: 1758-1776, DOI: 10.1007/s12011-011-8998-9

Ichikawa, H., Ichiyanagi, T., Xu, B., Yoshii, Y., Nakajima, M., Konishi, T. 2001. Antioxidant activity of anthocyanin extract from purple black rice. J. Med. Food 4: 211-218, DOI: 10.1089/10966200152744481

Jaiswal, P.C. 2004. Soil, plant and water analysis. New Delhi, Kalyani Publishers.

Krall, J.P., Edwards, G.E. 1992. Relationship between photosystem II activity and CO_2 fixation in leaves. Physiol. Plant. 86: 180-187, DOI: 10.1111/j.1399-3054.1992.tb01328.x

Krasensky, J., Jonak, C. 2012. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. J. Exp. Bot. 63: 1593-1608, DOI: 10.1093/jxb/err460

Lee, B.R., Jin, Y.L., Jung, W.J., Avice, J.C., Morvan-Bertrand, A., Ourrt, A., Park, C.W., Kim, T.H. 2008. Water deficit accumulates sugars by starch degradation-not by de novo synthesis-in white clover leaves (*Trifolium repens*). Physiol. Plant. 134: 403-411, DOI: 10.1111/j.1399-3054.2008.01156.x

Liang, Y.C., Sun, W., Zhu, Y-G., Christie, P. 2007. Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. Environ. Pollut 147: 422-428, DOI: 10.1016/j.envpol.2006.06.008

Lichtenthaler, H.K., Wellburn, A.R. 1985. Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. Biol. Soc. Trans. 11: 591-592, DOI: 10.1042/bst0110591

Lutts, S., Majerus, V., Kinet, J.M. 1999. NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. Physiol. Plant. 105: 450-458, DOI: 10.1034/j.1399-3054.1999.105309.x

Lyons, G., Stangoulis, J., Graham, R. 2003. High-selenium wheat: biofortification for better health. Nutr. Res. Rev. 16: 45-60, DOI: 10.1079/NRR200255

Mitra, J. 2001. Genetics and genetic improvement of drought resistance in crop plants. Curr. Sci. 80: 758-763

Müller, P., Li, X.P., Niyogi, K.K. 2001. Non-photochemical quenching. A response to excess light energy. Plant Physiol. 125: 1558-1566, DOI: 10.1104/pp.125.4.1558

Niedzwiedz-Siegien, I., Bogatek-Leszczynska, R., Come, D., Corbineau, F. 2004. Effects of drying rate on dehydration sensitivity of excised wheat seedling shoots as related to sucrose metabolism and antioxidant enzyme activities. Plant Sci. 167: 879-888, DOI: 10.1016/j.plantsci.2004.05.042

Noctor, G., Mhamdi, A., Foyer, C.H.A. 2014. The roles of reactive oxygen metabolism in drought: not so cut and dried. Plant Physiol. 164: 1636-1648, DOI: 10.1104/pp.113.233478

Owusu-Sekyere, A., Kontturi, J., Hajiboland, R., Rahmat, S., Aliasgharzad, N., Hartikainen, H., Seppänen, M.M. 2013. Influence of selenium (Se) on carbohydrate metabolism, nodulation and growth in alfalfa (*Medicago sativa* L.). Plant Soil 373: 541-552, DOI: 10.1007/s11104-013-1815-9

Pennanen, A., Xue, T., Hartikainen, H. 2002. Protective role of selenium in plant subjected to severe UV irradiation stress. J. Appl. Bot. 76: 66-76

Sperry, J.S. 2000. Hydraulic constraints on plant gas exchange. Agric. Forest Meteorol. 104: 13-23, DOI: 10.1016/S0168-1923(00)00144-1

Tardieu, F., Parent, B., Caldeira, C.F., Welcker, C. 2014. Genetic and physiological controls of growth under water deficit. Plant Physiol. DOI:10.1104/pp.113.233353.

Tennant, D. 1975. A test of modified line intersect method of estimating root length. J. Ecol. 63: 995-1001, DOI: 10.2307/2258617

Turakainen, M., Hartikainen, H., Seppänen, M.M. 2004. Effects of selenium treatments on patato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. J. Agric. Food Chem. 52: 5378-5382, DOI: 10.1021/jf040077x

Valliyodan, B., Nguyen, H.T. 2006. Understanding regulatory networks and engineering for enhanced

drought tolerance in plants. Curr. Opin. Plant Biol. 9: 1-7, DOI: 10.1016/j.pbi.2006.01.019

Verbruggen, N., Hermans, C. 2008. Proline accumulation in plants: a review. Amino Acids 35: 753-759, DOI: 10.1007/s00726-008-0061-6

Wahid, A., Ghazanfar, A. 2006. Possible involvement of some secondary metabolites in salt tolerance of sugarcane. J. Plant Physiol. 163: 723-730, DOI: 10.1016/j.jplph.2005.07.007

Wang, C.Q. 2011. Water-stress mitigation by selenium in *Trifolium repens* L. J. Plant Nutr. Soil Sci. 174: 276-282, DOI: 10.1002/jpln.200900011

Wang, Y.D., Wang, X., Wong, Y.S. 2012. Proteomics analysis reveals multiple regulatory mechanisms in response to selenium in rice. J. Proteomics 75: 1849-1866, DOI: 10.1016/j.jprot.2011.12.030

Yao, X.Q., Chu, J.Z., Wang, G.Y. 2009. Effects of drought stress and selenium supply on growth and physiological characteristics of wheat seedlings. Acta Physiol. Plant. 31: 1031-1036., DOI: 10.1007/s11738-009-0322-3

Yemm, E.W., Cocking, E.C. 1955. The determination of amino acids with ninhydrin. Analyst 80: 209-213, DOI: 10.1039/an9558000209

Yemm, E.W., Willism, A.J. 1954. The estimation of carbohydrates extracts by anthrone. Biochem. J. 57: 508-514, DOI: 10.1042/bj0570508

Zhou, S., Duursma, R.A., Medlyn, B.E., Kelly, J.W.G., Prentice, I.C. 2013 How should we model plant responses to drought? An analysis of stomatal and non-stomatal responses to water stress. Agric. Forest Meteorol. 182/183: 204-114, DOI: 10.1016/j.agrformet.2013.05.009