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ARTICLE

Different physiological and biochemical responses in maize hybrids subjected to drought stress at vegetative and reproductive stages

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ABSTRACT The growth and development of all plants are seriously limited by a variety of environmental stresses. In this study, responses in crop yield, physiological and biochemical traits were investigated in maize (*Zea mays* L.) hybrids such as SC704, SC700 (FAO 700), SC647 (FAO 600), SC500 (FAO 500), SC370 (FAO 300) and SC260 (FAO 200) subjected to drought stress induced by stopping irrigation at vegetative stage (4-5 leaves) and reproductive stage (anthesis). The results showed that drought stress had a significant effect on these characteristics. The yield and its components decreased considerably under water stress: the main factors that caused reduction of yield were the decrease in the number of grain per ear and 1000-grain weight. Reduction of yield in short maturity hybrids was higher than in other ones. Activity of superoxide dismutase (SOD) as well as permeability of plasma membrane increased significantly, but ascorbate peroxidase (APX) activity decreased under water stress. Maximum quantum efficiency of PSII (Fv/Fm) and performance index (PI) revealed a significant reduction in leaves in comparison with control plants. The content of soluble proteins in leaves reduced with increasing drought stress. These results suggested that water stress lead to the production of reactive oxygen species (ROS), which caused an increased membrane permeability and oxidative stress in the maize plants. Furthermore, maize hybrids with higher levels of antioxidant enzyme activities showed higher resistance to drought stress and produced higher yield under stress. So that SC704 and SC700 were the most tolerant, however, SC647 and SC500 were drought semi-tolerant to water stress, and SC370 and SC260 were the most sensitive.

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KEY WORDS

antioxidant enzymes
drought stress
lipid peroxidation
maize
maximum quantum efficiency of PSII

Abbreviations

reactive oxygen species (ROS), superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical (HO·), hydrogen peroxide (H_2O_2), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), relative water content (RWC), maximum quantum efficiency of PSII (Fv/Fm), photosystem II (PSII), performance index (PI), field capacity (FC), nitroblue tetrazolium (NBT), plant efficiency analyser (PEA), fresh weight (FW)

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Introduction

Among the crop and forage plants, maize (*Zea mays* L.) is important for global food security (Gerpacio and Pingali 2007). Maize ranks third after wheat and rice for its grain production in Iran, additionally, maize is cultivated two times during an each year in Iran (spring and summer). Enough moisture is essential for normal plant growth and for the completion of its life cycle (Zhu 2002). Insufficient moisture conditions, resulting to water stress, usually exist in semiarid regions which considerably limits plant growth and crop productivity (Wang et al. 2005; Alahdadi et al. 2011). Globally, drought stress affects 20 to 25% of the planting areas of maize (Golbashy et al. 2010).

Most of the hurt on plants is connected to oxidative damage at the cellular level under abiotic stress, such as drought (Sharma et al. 2012). Oxidative stress is induced within the plants cell owing to higher leakage of electrons during photosynthetic and respiratory processes (Das and Roychoudhury 2014).

The ROS like as superoxide anion radical ($O_2^{\cdot -}$), hydroxyl radical (HO·) and hydrogen peroxide (H_2O_2) can cause severely injury to membrane lipids, proteins and DNA nicking, that finally leads to cell death (Mittler 2002; Simova-Stoilova et al. 2008). During optimal and best growth conditions, balance between ROS information and consumption is tightly controlled by antioxidant defense systems of plants. Catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) are antioxidants, which play key role in the defense against ROS (Racchi 2013). The antioxidant protection is complex and highly partitioning in plant cells. The SOD is a family of enzymes catalyzing the dismutation of $O_2^{\cdot -}$ to H_2O_2 in organelles (*i.e.* cytosol), and also the latter is broken down by CAT and peroxidase (POD) enzymes (Sharma et al. 2012). The relation between drought stress and enzymatic antioxidant systems has been studied in some plant species (*e.g.*, Ge et al. 2006). Believed, that tolerance to drought stress depends on the high activity of SOD, CAT and POD enzymes in sensitive plants maize.

The impact of the drought on given plant species depends on variety, severity and duration of the stress as well as on the development stage (Simova-Stoilova et al. 2008). It is possible that genotypes might respond differently under sever water stress at different growth stage. So, that drought stress results in several changes in the subjected plants, including leaf relative water content (RWC), electrolytes leakage of plasma membrane, soluble protein content, photosynthetic pigments, carotenoids, etc., which in turn decrease the efficiency expected of photosynthesis rate and photo-assimilate production, and finally reduction of crop yield (Lonbani and Arzani 2011; Rosales et al. 2012; Barbosa et al. 2015).

The main aim of this study is to understand the possible responses and adaptation of maize plants to drought and the function of maximum quantum efficiency of PSII and some physiological and biochemical processes, as well as changed yield and its components. By this study, the development of scientific strategies in order to alleviate negative effects of climatic change such as water deficiency may be discussed.

Materials and Methods

Plant materials and experimental details

This pot experiment was carried out using factorial arrangement, based on randomized design with four replications, and carried out in Campus of Agricultural and Natural Resource of the Razi University (Kermanshah) at west of Iran (47º,9 /E; 34º,21 /N; with 1319 m elevated from sea level) during the growing season from 2012. Plastic pots (diameter of 30 cm and height of 50 cm) were used for the implantation of maize hybrids. The pots were filled with 11.5 kg of silty-clay soil (Table 1). Before filling the pots, 15 g N, 0.33 g P and 9.1 g K was added to the soil. Another 0.9 g N per pot was added top dressed at five to six leaves stage. Each pot (2 plants per pots) was irrigated up to 80 ± 5% of field capacity (FC) before the four-leaf stage, and weeds were controlled manually. The experiment comprised irrigation treatments as control (WW, well watered, 80 ± 5% FC), early water shortage {(WS1 50 ± 5% FC at the vegetative growth stage (4-5 leaves) and re-watering after 3 weeks)}, and late water shortage {(WS2 50 ± 5% FC at the reproductive stage (anthesis) and re-watering after 3 weeks)}, and six maize hybrids *i.e.*, SC704 (FAO 700, Late maturity), SC700 (FAO 700, Late maturity), SC647 (FAO 600, Medium-Late maturity), SC500 (FAO 500, Medium-Late maturity), SC370 (FAO 300, Early-Medium maturity) and SC260 (FAO 200, Early maturity). These hybrids were chosen because of their contrasting grain and forage yield productivity and existence of the highest area under cultivation in the west of Iran, especially Kermanshah province. The maize hybrids have been provided from Seed and Plant Improvement Institute (SPII) of Karaj, Iran. The vegetative stage stress was started on 8 June 2012 and the reproductive stage stress on 19 August 2012 for 3 weeks each. Plants of WS1 treatment were harvested on 7 September 2012 and WS2 treatment on 16 September 2012. The climate based on Domarten classification is semi-arid cold in the test area. Mean temperatures and rainfall during the crop season are presented in Table 2. Antioxidant enzyme activities, soluble

Table 1. Physical and chemical characteristics of the soil used in the pot experiment.

Soil type	pH	†OC	K	Ca	Mg	P	As	Cd	Pb	Zn
		(%)	mg kg^{-1}							
Silty-clay	6.8	2.1	221	5717	200	131	17.8	0.414	29.1	1.87

†OC: organic carbon. Note: Available nutrients were determined in extracts obtained by Mehlich III procedure (Zbíral 2000).

Table 2. Rainfall, temperature and evaporation at the site of experiment during the growing season from 2012.

Month	Rainfall (mm)	Temperature (°C) Minimum	Maximum	Mean	Evaporation (mm)
May	25.2	19.3	26.5	17.8	121
June	0	14.2	23.7	23.9	305
July	0	17.0	36.9	27.0	361
August	0	19.0	39.0	29.0	368
September	0	15.0	36.0	25.5	285

Source: Meteorological Organization, Kermanshah, Iran.

protein content and electrolyte leakage were determined always in the youngest plant leaves at every stage.

Enzyme extraction

Fresh leaf materials (0.2 g), homogenized in liquid nitrogen were transferred into 15 ml tubes, after which 2.5 ml of the extraction buffer (0.1 M Tris, pH 7.8, and 30% glycerol) was added to the samples. They were centrifuged at 15 000 g for 15 min at 4 °C, and the supernatant was used for enzyme assays.

Antioxidant enzymes assay

The activity of superoxide dismutase (SOD, EC 1.15.1.1) following the method of Fridovich (1974), was assayed by measuring its ability to inhibit the photochemical decrease of nitroblue tetrazolium (NBT) at 560 nm by Elisa. One unit of SOD activity was defined as the value of enzyme, which caused 50% inhibition of photochemical reduction of NBT. The SOD activity was expressed as the SOD units mg^{-1} protein.

For assay of the ascorbate peroxidase (APX, EC 1.11.1.11) activity, the rate of hydrogen peroxide-dependent oxidation of ascorbic acid was determined in a reaction mixture. The oxidation rate of ascorbic acid was estimated by following the declined in absorbance at 290 nm by a microplate spectrophotometer (PowerWave XS, BioTek, USA) for 10 min by Nakano and Asada (1981) method. The enzyme activity was expressed as APX µmol min^{-1} mg^{-1} protein.

Total soluble protein measurements

The measurement of the total soluble proteins was performed in 100 mg of leaf powder incubated in 5 ml of extraction buffer (Tris-HCl at 25 mM and pH 7.6). The quantification of the total soluble proteins according to Bradford (1976), was carried out at 595 nm by Elisa (Power wave XS, Bio Tek, USA) and with albumin bovine (BSA, Sigma) used as standard.

Membrane permeability measurements

Membrane permeability of leaves was measured by electrolyte leakage (Dhindsa et al. 1981); leaves were cut and placed in a beaker containing 20 ml of distilled water and after 3 h at room temperature (25 °C) the conductivity (HI 2004, Hanna Instruments, Padova, Italy) of the solution was measured.

Maximum quantum efficiency of PSII and performance index measurements

Maximum quantum efficiency of PSII (Fv/Fm) and performance index (PI) was recorded on the two of youngest leaves of each plant in pot by using the time-resolving portable fluorimeter (PEA, Hansatech Instrument, Kings Lynn, UK). The leaf clips were placed on the leaves 20 min prior to the measurements to provide dark adaptation. After that, samples were illuminated with continuous red light (wavelength in peak 650 nm, spectral line half-width 22 nm). The light was provided by an array of three light-emitting diodes. The light pulse intensity used was 3500 µmol m^{-2} s^{-1} and the duration of the light pulse was 2 s. Leaf segment measurements were performed in the middle part of a leaf blade, away from the main leaf vein. The Fv/Fm showed that the maximal quantum yield of photochemistry in dark-adapted state and PI or performance index expresses, an ability of the plant to avoid drought and to maintain its physiological activity at a certain level. This performance index (PI) combines criteria of structure and function: it reflects the fraction of non-photochemical phenomenon (fluorescence and heat dissipation), when the majority of the PSII reaction centers are open for maximal photon absorption (Strasser and Strasser 1995; Strasser et al. 1999). Fv/Fm was calculated according to the equation:

Fv/Fm = (Fm - F0) / Fm, where, Fm represent maximal fluorescence yield of dark-adapted sample with all PSII centers closed, and F0 represent minimal fluorescence yield of dark-adapted sample with all PSII centers open.

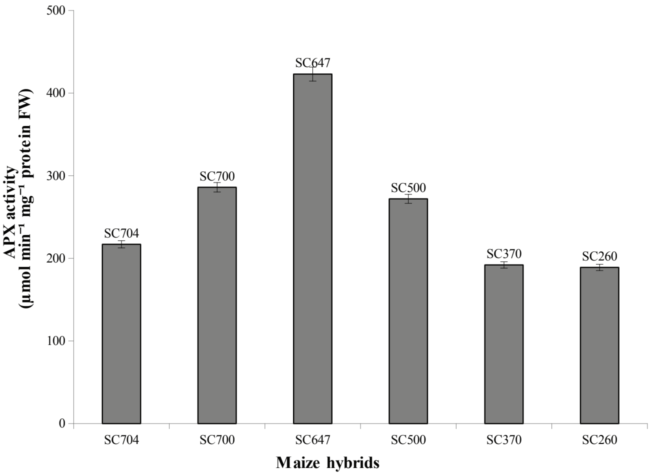

Figure 1. Effect of early water shortage (WS1: 50% FC at vegetative stage for 3 weeks) on ascorbate peroxidase (APX) enzyme activity of maize hybrids. The values are mean ± standard error. Means in the same column followed by the same letter are not significantly ($P < 0.05$) different according to Duncan's Multiple Range test.

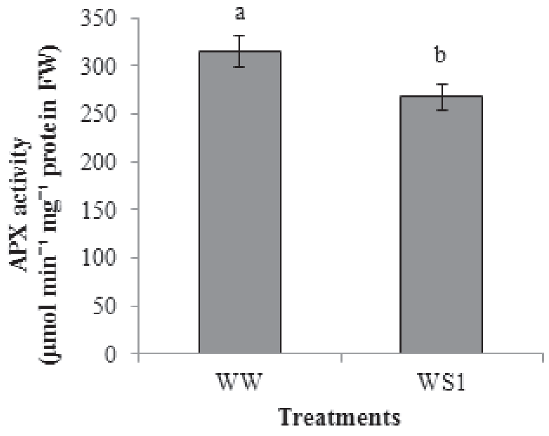

Figure 2. Ascorbate peroxidase (APX) enzyme activity of maize hybrids at drought stress for 3 weeks during vegetative stage; WW: well watered (80% FC) and WS1: 50% FC at vegetative stage. The values are mean ± standard error. Means in the same column followed by the same letter are not significantly ($P < 0.05$) different according to Duncan's Multiple Range test

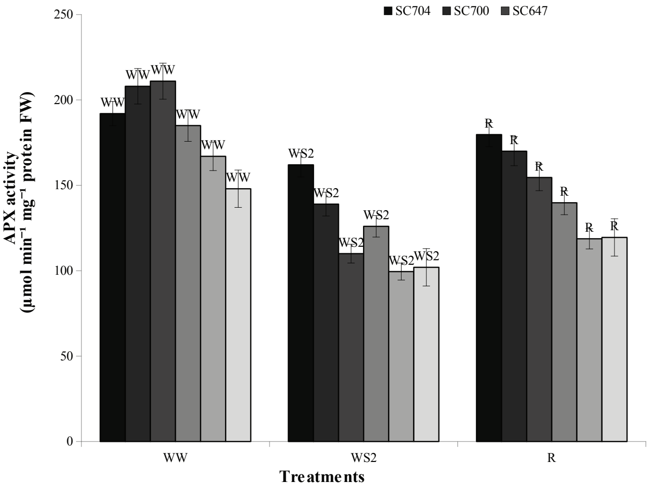

Figure 3. Ascorbate peroxidase (APX) enzyme activity of maize hybrids at drought stress for 3 weeks during the reproductive stage, and following re-watering 4 weeks; WW: well watered (80% FC), WS2: 50% FC at reproductive stage, and R: re-watered treatment after stress for 4 weeks. The values are mean ± standard error. Means in the same column followed by the same letter are not significantly ($P < 0.05$) different according to Duncan's Multiple Range test.

Measuring yield and its components

For measuring the number of grain per row, number of row per ear, 1000-grain weight and dry weight of ear (grain yield), 10 plants harvested from each of the treatments (five pots) were measured.

Statistical analysis

Significant of data was tested by analysis of variance (ANOVA) and Duncan`s multiple range test (DMRT) at $P < 0.05$ by using MSTAT-C software (Ver. 1.42) and SAS software (Ver. 9.1). The figures were drawn using Excel software (Ver. 10.0).

Results

Antioxidant enzymes activities

The result showed that the APX enzyme activity in leaves of maize altered under water stress at different phenology stages (Figs. 1-3). It significantly decreased in leaves of all maize hybrids ($P < 0.01$) almost 34.8% at the first stage stress (stress at reproductive stage) and then enhanced gradually following re-watering though level of increase varied among hybrids. The amount of rudimentary reduction was associated with intensity and period of drought stress. Highest APX activity was observed in SC647 (FAO 600) than the other maize hybrids under water stress at vegetative stage (Fig. 1). Under re-watering at reproductive stage, APX activity increased considerably ($P < 0.01$) in all hybrids; however the SC370 and SC260 (FAO 300 and 200) remained unaffected in re-watering stage (Fig. 3).

SOD enzyme activity raised significantly ($P < 0.01$) under stress at all stages in all maize hybrids (Table 3). The highest increase in SOD was obtained in SC704 (FAO 700) followed

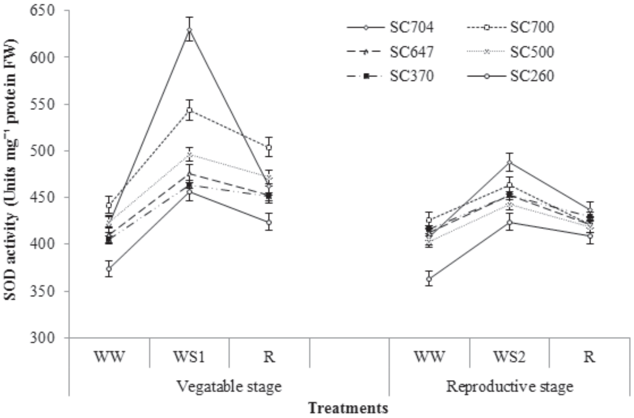

Figure 4. Superoxide dismutase (SOD) enzyme activity of maize hybrids at drought stress for 3 weeks during vegetative stage and reproductive stage, and following re-watering for 4 weeks; WW: well watered (80% FC), WS1: 50% FC at vegetative stage, WS2: 50% FC at reproductive stage, and R: re-watering after stress for 4 weeks. The values are mean ± standard error.

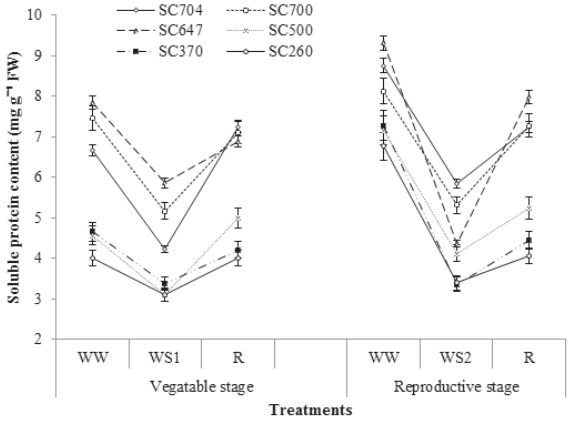

Figure 5. Soluble protein content of maize hybrids at drought stress for 3 weeks during the vegetative stage and reproductive stage, and following re-watering 4 weeks; WW: well watered (80% FC), WS1: 50% FC at vegetative stage, WS2: 50% FC at reproductive stage, and R: re-watered treatment after stress for 4 weeks. The values are mean ± standard error.

by SC700 (FAO 700) maize hybrids and SC260 (FAO 200) had the lowest SOD activity during water stress at the vegetative stage and reproductive stage. At recovery after water stress at vegetative and reproductive stages the value of SOD activity decreased (Fig. 4).

Soluble protein content

The effects of water stress on the soluble protein content in maize leaves are shown in Figure 5. At two different growth stages (vegetative stage and reproductive stage), the soluble protein content in leaves first decreased drastically and then increased during the later and by re-watering of maize development. Leaf soluble protein content was changed in altogether hybrids.

Membrane permeability

During maize growth, electrolyte leakage in leaves increased from vegetative growth to the early tasseling period from 45.6 to 75.4% (data not shown), and electrolyte leakage of plasma membrane in maize plants increased with decreasing soil water content at combination of vegetative and reproductive stages (Figs. 6-8). Highest membrane stability was observed in SC704 and SC700 (FAO 700) during drought stress, but SC370 and SC260 (FAO 300 and 200) maize hybrids had the

Table 3. The analysis of variance for the effect of drought stress at vegetative stage and reproductive stage on ascorbate peroxidase (APX) and superoxide dismutase (SOD) activity, soluble protein content (SPC), membrane permeability (MP), maximum quantum efficiency of PSII (Fv/Fm), and performance index (PI) of maize hybrids.

Source of variation	df	Mean squares					
		APX	SOD	SPC	MP	Fv/Fm	PI
Vegetative stage							
Hybrids (H)	5	*	*	**	**	**	**
Drought stress (DS)	2	**	**	*	*	**	**
H × DS	10	ns	**	*	ns	**	**
CV (%)	-	12.5	9.11	11.2	14.6	8.51	18.4
Reproductive stage							
Hybrids (H)	5	**	*	*	**	**	**
Drought stress (DS)	2	**	**	*	**	**	**
H × DS	10	**	*	*	**	**	**
CV (%)	-	11.9	10.1	10.7	17.6	11.4	16.9

ns, * and **, non-significant and significant at the 5 and 1% levels of probability, respectively.

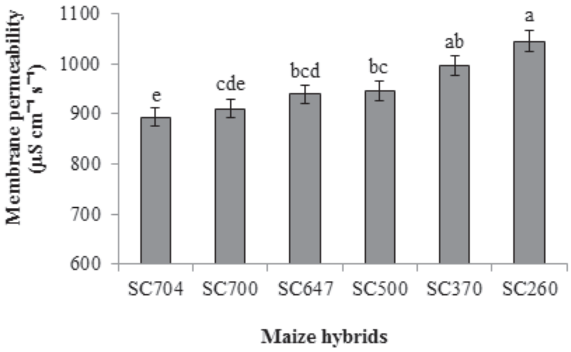

Figure 6. Effect of early water shortage (WS1: 50% FC at vegetative stage for 3 weeks) on membrane permeability of maize hybrids. The values are mean ± standard error. Means in the same column followed by the same letter are not significantly ($P < 0.05$) different according to Duncan's Multiple Range test.

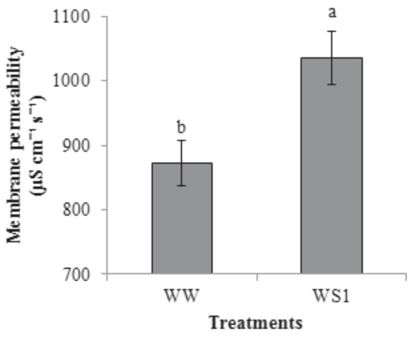

Figure 7. Membrane permeability of maize hybrids at drought stress for 3 weeks during vegetative stage; WW: well watered (80% FC), and WS1: 50% FC at vegetative stage. The values are mean ± standard error. Means in the same column followed by the same letter are not significantly ($P < 0.05$) different according to Duncan's Multiple Range test.

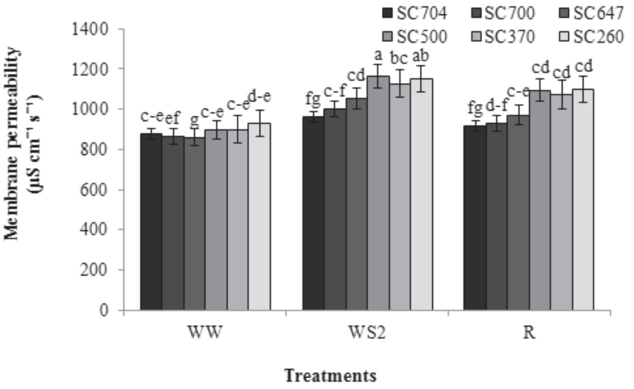

Figure 8. Membrane permeability of maize hybrids at drought stress for 3 weeks during reproductive stage and both vegetative and reproductive stages, and following re-watering for 4 weeks; WW: well watered (80% FC), WS2: 50% FC at reproductive stage, and R: re-watering after stress for 4 weeks. The values are mean ± standard error. Means in the same column followed by the same letter are not significantly ($P < 0.05$) different according to Duncan's Multiple Range test.

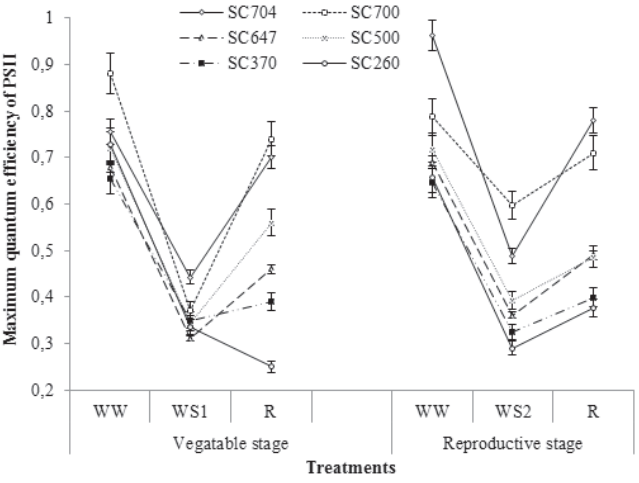

Figure 9. Maximum quantum efficiency of PSII (Fv/Fm) of maize hybrids at drought stress for 3 weeks during vegetative stage and reproductive stage, and following re-watering for 4 weeks; WW: well watered (80% FC), WS1: 50% FC at vegetative stage, WS2: 50% FC at reproductive stage, and R: re-watering after stress for 4 weeks. The values are mean ± standard error.

lowest membrane stability under water stress (Fig. 8). Under well watered condition, electrolyte leakage was similar in all hybrids. This indicates that drought-tolerant genotypes have higher hereditary and induced capability of plant protection against oxidative damage caused by drought treatment.

Maximum quantum efficiency of PSII and performance index

Results showed that maximum quantum efficiency of PSII and performance index was reduced in maize plants which received less water at several growth stages (Figs. 9-10). Among the studied hybrids, SC704 and SC700 (FAO 700) demonstrated higher maximum quantum efficiency of PSII than other hybrids. During the growth and development of re-watering a gradual increase of efficiency of PSII and performance index occurred.

Yield and its components

Results indicated that drought stress had a significant effect on grain yield and its components of maize plants (Table 4). Agronomic traits such as 1000-grain weight, number of grain per row and number of row per ear and other traits declined in

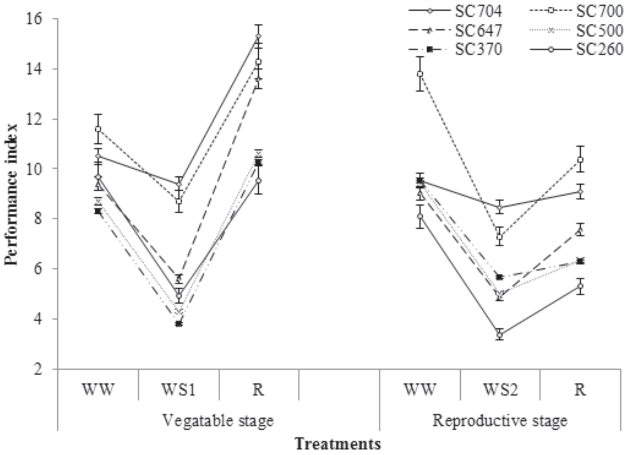

Figure 10. Performance index of maize hybrids at drought stress for 3 weeks during vegetative stage and reproductive stage, and following re-watering for 4 weeks; WW: well watered (80% FC), WS1: 50% FC at vegetative stage, WS2: 50% FC at reproductive stage, and R: re-watering after stress for 4 weeks. The values are mean ± standard error.

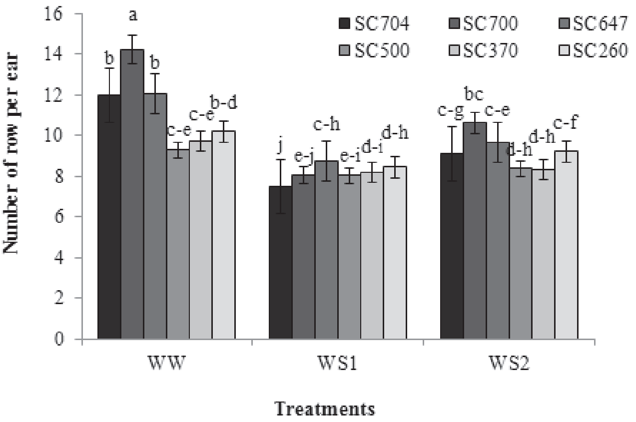

Figure 11. Number of row per ear of maize hybrids after drought stress for 3 weeks during vegetative stage and reproductive stage, and following re-watering for 4 weeks; WW: well watered (80% FC), WS1: 50% FC at vegetative stage, and WS2: 50% FC at reproductive stage. The values are mean ± standard error. Means in the same column followed by the same letter are not significantly (P < 0.05) different according to Duncan's Multiple Range test.

water-stressed plants (Figs. 11-14). Number of grain per row was the highest in SC704, SC700 and SC647 (FAO 700 and 600), while it was the lowest in SC500, SC370 and SC260 (FAO 500, 300 and 200) under well watered and water stress conditions (Fig. 12). Yield of maize showed high reduction with decreasing soil water content during the vegetative and reproductive stages (Fig. 14). When compared to the well watered treatment, grain yield and the number of grain per row declined (Fig. 12). Moreover, ear length, ear diameter (data not shown) and 1000-grain weight, also decreased in drought treatments (Fig. 14). Grain yield was the highest in SC704 (FAO 700) while it was the lowest in SC260 (FAO 200) under well watered and water stress conditions (Fig. 14). When subjected to drought stress, plant growth and yield reduced significantly in all tested hybrids. Under drought stress condition, 1000-grain weight decreased by 42.7%, compared to their respective plants under well watered conditions. The main factors that caused reduction of yields were the decrease of the number of grain per ear and 1000-grain weight.

Discussion

APX enzyme activity significantly decreased with decreasing irrigation from 80% to 50% FC during the vegetative (WS1) and reproductive (WS2) stages (Figs. 2-3). Indeed, the flexibility for fast up-regulation of the antioxidant system was found to be closely associated with reduced oxidative injury and, therefore, drought tolerance in various species and different genotypes of one species (Sofo et al. 2005; Aganchich et al. 2009; Wang et al. 2009; Bies and Patakas 2012). When the plants suffered from drought, the whole defensive system required to be activated in order to resist the ROS injury. Antioxidant enzymes or other antioxidants cannot resist singly the damages. Uniform distribution of soil water deficit, is an opportunity for precise comparison of varieties (Simova-Stoilova et al. 2008; Goodarzian-Ghahfarokhi et al. 2015). Jiang and Ren (2004) reported that the activity of POD, SOD and APX in many plants were affected by environmental

Table 4. The analysis of variance for the effect of drought stress at different growth stage on number of row per ear, number of grain per row, 1000-grain weight and grain yield of maize hybrids.

Source of variation	df	Mean squares			
		Number of row per ear	Number of grain per row	1000-grain weight	Grain yield
Hybrids (H)	5	**	**	*	*
Drought stress (DS)	2	*	**	**	*
H × DS	10	**	**	**	**
CV (%)	-	14.6	12.4	16.1	15.8

ns, * and **, non-significant and significant at the 5 and 1% levels of probability, respectively.

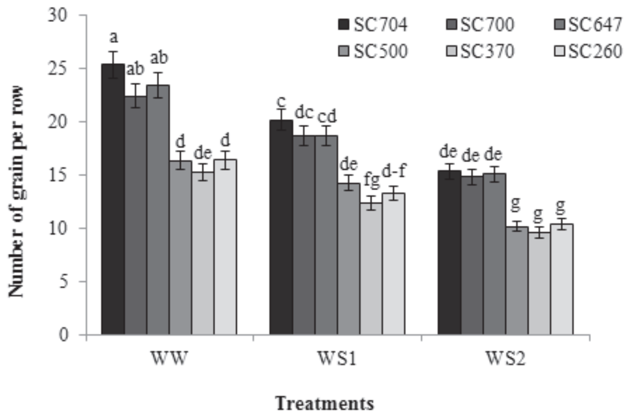

Figure 12. Number of grain per row of maize hybrids after drought stress for 3 weeks during vegetative stage and reproductive stage, and following re-watering for 4 weeks; WW: well watered (80% FC), WS1: 50% FC at vegetative stage, and WS2: 50% FC at reproductive stage. The values are mean ± standard error. Means in the same column followed by the same letter are not significantly (P < 0.05) different according to Duncan's Multiple Range test.

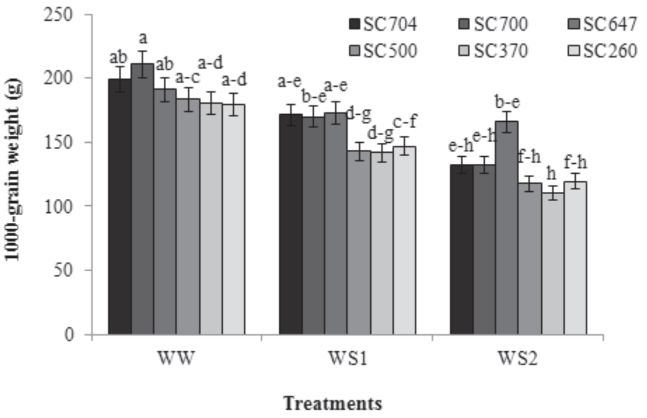

Figure 13. 1000-grain weight of maize hybrids after drought stress for 3 weeks during vegetative stage and reproductive stage, and following re-watering for 4 weeks; WW: well watered (80% FC), WS1: 50% FC at vegetative stage, and WS2: 50% FC at reproductive stage. The values are mean ± standard error. Means in the same column followed by the same letter are not significantly (P < 0.05) different according to Duncan's Multiple Range test.

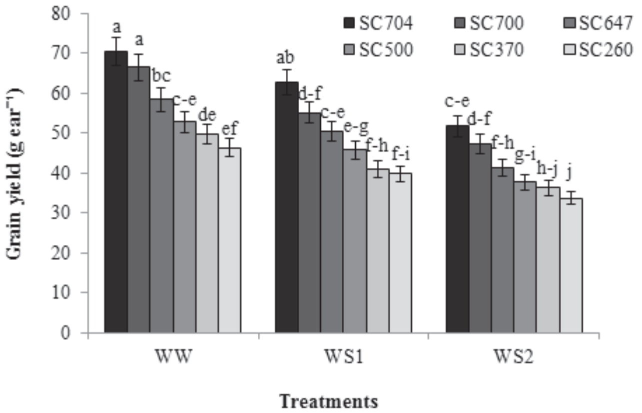

Figure 14. Grain yield of maize hybrids after drought stress for 3 weeks during vegetative stage and reproductive stage, and following re-watering for 4 weeks; WW: well watered (80% FC), WS1: 50% FC at vegetative stage, and WS2: 50% FC at reproductive stage. The values are mean ± standard error. Means in the same column followed by the same letter are not significantly (P < 0.05) different according to Duncan's Multiple Range test.

stresses, specially drought stress. Several researches have shown that the grade of injury caused by drought was negatively correlated with the improvement of activity of APX in genotypes of maize plants (Ana Carolina et al. 2009; Sharma et al. 2012). Sun et al. (2003) stated that the activity of antioxidant enzymes such as SOD and POD increased, while that of APX declined under water stress. Some studies have got the similar results in different plants (Wang et al. 2002; Ge et al. 2006). These variations and differences may be due to other experiments using different experimental method, *i.e.* unusual materials and different methods. Different activities of ROS scavenging enzymes including SOD, APX and CAT below stress at different growth stages have additionally been reported by Ezzat-Ollah et al. (2007) and Simova-Stoilova et al. (2008). Higher accumulation of antioxidant enzymes like SOD and APX and fewer quantity of electrolyte leakage in tolerant and susceptible maize hybrids had been regularly reported under water stress (Wang et al. 2002). In this case, Chaves and Oliveira (2004) announced that decrease of APX activity may be a result of the inhibition of photosynthesis and the high hydrogen peroxide production level under drought. It is well documented that the augmentation of lipid peroxidation inhibited the APX activity, therefore its protective system was lost and the membrane injury more worsened.

Results indicated that drought stress also had a significant effect (P < 0.01) on SOD enzyme activity in the leaves of maize hybrids (Table 3). In present study, under stress at vegetative and reproductive growth stages, the highest APX and SOD activities were observed in SC704 and followed by SC700 and SC647 hybrids (Figs. 1-4). Based on above mentioned reports, these hybrids attempted to be tolerant by possessing comparatively higher antioxidant activities. In the meantime, amount of altered SOD activity was related to drought severity, drought duration and growth stage (Ge et al. 2006). The change of enzyme activity and total soluble protein content were very diverse at different growth periods because of the interval of drought stress (Fig. 5). This emphasizes the importance of critical growth stages for water supply in all plants.

It seems that the initial decrease of total soluble protein content pending drought stress was due to a severe decrease in photosynthesis. Photosynthesis reduced (Chaves et al. 2009) and the assimilates for protein synthesis were not provided, therefore, protein synthesis dramatically descended or even stopped in drought stress. The amount of protein raised at later stage which was probably due to the expression of new stress proteins (*e.g.*, dehydrins). The initial decrease and subsequently increase of total protein content at these conditions was similar to the finding of Ge et al. (2006) and Mohammadkhani and Heidari (2008) in maize, Lqbal and Bano (2009) in wheat, Costa et al. (2011) in cowpea, and Hossain and Komatsu (2014) in soybean plants. Enhanced ROS under drought can induce lipid peroxidation and membrane injury eventually leading to cell death in organelles (Mittler 2002).

Under water stress condition, electrolyte leakage increased considerably in all hybrids, but decreased at re-watering (Figs. 6-8). There is possibility that the defense systems were inefficient including the protection of membrane stability inverse oxidation induced by two levels of water stress in this experiment. In previous literature, several reports demonstrated similar results induced by drought, that lipid peroxidation enhanced in maize (Ge et al. 2006; Zhu et al. 2011). In our study, degree of damage of membranes varied in genotypes under different stage of water stress.

The maximum quantum efficiency of PSII (Fv/Fm) at nearly full irrigation ranged between 0.7 and 0.8 (Carvalho et al. 2011; Saeidi et al. 2015). This ratio is close to the values reported for most plant species in stress conditions. Recent examinations have emphasized that changes in PSII fluorescence may result from the harm in the reaction center, including non-radiate dissipation or increased excitation transfer to PSII (Ashraf and Harris 2013). Lepedus et al. (2012) exhibited that the leaves of maize seedlings exposed to drought had lower maximal efficiency of PSII photochemistry compared with control seedlings. Also, Ashraf and Harris (2013) reported that decrease in Fv/Fm ratio could be associated with an enhanced dissipation of energy in the PSII antenna. In our case, water stress applied at vegetative-and reproductive stages significantly decreased maximum quantum efficiency of PSII (Fv/Fm) and performance index (PI) in most of the hybrids (Figs. 9 and 10). Our findings indicated that SC704 and SC700 (FAO 700) had higher maximum quantum efficiency of PSII followed by SC647 and SC500, and whereas SC370 and SC260 had the lowest of maximum quantum efficiency of PSII (Fig. 9). These low values were probably due to a greater degradation PSII reaction centers (Carvalho et al. 2011; Ashraf and Harris 2013) and/or to a fewer reparation amount of D1 and other proteins (Nishiyama et al. 2006).

In general, the economic yield of maize decreased considerably under water stress, namely the number of grain per ear and 1000-grain weight (Figs. 11-14). These results are in agreement with the results reported by Saeidi et al. (2015), that the water stress significantly provoked a reduction of grain yield by decreasing the number of grains per spike. Furthermore, this lower yield under drought stress was related to SC704 and SC700 (FAO 700) hybrids (Figs. 11-14). It means that these genotypes with increased antioxidant enzyme activities showed greater tolerance against ROS. Under drought stress, the accumulation of assimilates was reduced and the translocation and re-distribution of these were also affected. This may have been one of the main reasons that the capacity of the sink organs attenuated, components of the yield deteriorated markedly and the grain yield and the biomass sharply dropped.

Conclusion

Our results clearly showed that maize hybrids differently replied to soil water stress at different stages in term of physiological mechanisms, implying that they have different soil water-stress thresholds. Soil water-stress threshold for various hybrids can also be influenced via developmental course and enzymes activities (*e.g.*, APX and SOD). In conclusion, genotypic differences in drought tolerance were mainly attributed to the ability of maize plants to trigger antioxidant defense ability of genotypes to induce various antioxidant responses under water stress. SC704 and SC700 hybrids (FAO 700) showed to have better drought tolerance than the others, possessing higher antioxidant enzyme activities which resulted in lower oxidative injury. The variation found amongst the genotypes tested suggests that the existence of valuable genetic resources for crop improvement is in relation with drought tolerance. As well as, maximum quantum efficiency of PSII and lipid peroxidation within the youngest of the leaves can be used as markers of water deficit tolerance in maize hybrids.

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References

Aganchich B, Wahbi S, Loreto F, Centrito M (2009) Partial root zone drying: regulation of photosynthetic limitations and antioxidant enzymatic activities in young olive (*Olea europaea*) saplings. Tree Physiol 29:685-696.

Alahdadi I, Oraki H, Parhizkar Khajani F (2011) Effect of water stress on yield and yield components of sunflower hybrids. Afr J Biotechnol 10:6504-6509.

Ana Carolina F, Vasconcelos D, Xunzhong Z, Ervin E (2009) Enzymatic antioxidant responses to bio-stimulants in maize and soybean subjected to drought. Sci Agric, Piracicaba Braz 66(3):395-402.

Ashraf M, Harris PJC (2013) Photosynthesis under stressful environments: An overview. Photosynthetica 51(2):163-190.

Barbosa AM, Guidorizi KA, Catuchi TA, Marques TA, Ribeiro RV, Souza GM (2015) Biomass and bioenergy partitioning of sugarcane plants under water deficit. Acta Physiol Plant 37(142):1-8.

Bies A, Patakas A (2012) Relative contribution of photoprotection and anti-oxidative mechanisms to differential drought adaptation ability in grapevines. Environ Exp Bot 78:173-183.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Ann Biochem 72:248-254.

Carvalho RC, Cunha A, Marques J (2011) Photosynthesis by six Portuguese maize cultivars during drought stress and recovery. Acta Physiol Plant 33:359-374.

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann Bot 103:551-560.

Chaves MM, Oliveira MM (2004) Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. J Exp Bot 55:2365-2384.

Costa RCL, Lobato AKS, Silvera JAG, Laughinghousevi DH (2011) ABA mediated proline synthesis in cowpea leaves exposed to water deficiency and rehydration. Turk J Agric For 35(3):309-317.

Das K, Roychoudhury A (2014) Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Front Environ Sci 2:1-13.

Dhindsa R, Plumb-Dhindsa P, Thorpe T (1981) Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot 32:93-101.

Ezzat-Ollah E, Shakiba MR, Mahboob SA, Hoshang A, Mahmood T (2007) Water stress, antioxidant enzyme activity and lipid peroxidation in wheat seedling. Inter J Food Agric Environ 5(1):149-153.

Fridovich I (1974) Superoxide dismutases. Adv Enzymol Relat Areas Mol Biol 41:35-97.

Ge TD, Sui FG, Bai LP, Lu YY, Zhou GS (2006) Effects of water stress on the protective enzyme activities and lipid peroxidation in roots and leaves of summer maize. Agri Sci China 5(4):291-298.

Gerpacio VR, Pingali PL (2007) Tropical and subtropical maize in Asia: production systems, constraints and research priorities. CIMMYT, Mexico, pp. 93.

Golbashy M, Ebrahimi M, Khavari Khorasani S, Choukan R (2010) Evaluation of drought tolerance of some corn (*Zea mays* L.) hybrids in Iran. Afr J Agric Res 5:2714-2719.

Goodarzian-Ghahfarokhi M, Mansurifar C, Taghizadeh-Mehrjardi R, Saeidi M, Jamshidi AM, Ghasemi E (2015) Effects of drought stress and re-watering on antioxidant systems and relative water content in different growth stages of maize (*Zea mays* L.) hybrids. Arch Agron Soil Sci 61(4):493-506.

Hossain Z, Komatsu S (2014) Potentiality of soybean proteomics in untying the mechanism of flood and drought stress tolerance. Proteomes 2:107-127.

Jiang HF, Ren XP (2004) The effect on SOD activity and protein content in groundnut leaves by drought stress. Acra Agro Sin 30:169-174.

Lepedus H, Ivan B, Vlatka J (2012) Chlorophyll fluorescence analysis of photosynthetic performance in seven maize inbred lines under water-limited conditions. Peri Biol 114:73-76.

Lonbani M, Arzani A (2011) Morphophysiological traits associated with terminal drought stress tolerance in triticale and wheat. Agric Res 9:315-329.

Lqbal S, Bano A (2009) Water stress induced changes in antioxidant enzymes, membrane stability and seed protein profile of different wheat accessions. Afr J Biotechnol 8(23):6576-6587.

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405-410.

Mohammadkhani N, Heidari R (2008) Effects of drought stress on soluble proteins in two maize varieties. Turk J Biol 32:23-30.

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22(5):867-880.

Nishiyama Y, Allakverdiev SI, Murata N (2006) A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II. Biochim Biophys Acta 1157:742-749.

Racchi M (2013) Antioxidant defenses in plants with attention to *Prunus* and *Citrus* spp. Antioxidants 2(4):340-369.

Rosales MA, Ocampo E, Rodríguez-Valentin R, Olvera-Carrillo Y, Acosta-Gallegos J, Covarrubias AA (2012) Physiological analysis of common bean (*Phaseolus vulgaris* L.) cultivars uncovers characteristics related to terminal drought resistance. Plant Physiol Bioch 56:24-34.

Saeidi M, Ardalani S, Jalali-Honarmand S, Ghobadi ME, Abdoli M (2015) Evaluation of drought stress at vegetative growth stage on the grain yield formation and some physiological traits as well as fluorescence parameters of different bread wheat cultivars. Acta Biol Szeged 59(1):35-44.

Sharma P, Bhaushan Jha A, Shanker Dubey R, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot 2012:1-26 (ID 217037).

Simova-Stoilova L, Demirevska K, Petrova T, Tsenov N, Feller U (2008) Antioxidative protection in wheat varieties under severe recoverable drought at seedling stage. Plant Soil Environ 54:529-536.

Sofo A, Dichio B, Xiloyannis C, Masia A (2005) Antioxidant defences in olive trees during drought stress: changes in activity of some antioxidant enzymes. Funct Plant Biol 32:45-53.

Strasser BJ, Strasser RJ (1995) Measuring fast fluorescence transients to address environmental questions: the JIP test. In: Mathis P (ed.), Photosynthesis: From Light to Biosphere 5:977-980, Kluwer Academic Publisher, Dordrecht, The Netherlands.

Strasser RJ, Srivastava A, Tsimilli-Michael M (1999) The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Mohanty M, Yunus U, Pathre P. (ed.), Probing Photosynthesis: Mechanisms, Regulation and Adaptation. Taylor and Francis, London, pp. 445-483.

Sun CJ, Liu ZG, Jing YD (2003) Effect of water stress on activity and isozyme of the major defence-enzyme in maize leaves. J Maize Sci 11:63-66.

Wang FZ, Wang QB, Kwon SY, Kwak SS, Su WA (2005) Enhanced drought tolerance of transgenic rice plants expressing a pea manganese superoxide dismutase. J Plant Physiol 162:465-472.

Wang J, Li DQ, Gu LS (2002) The response to water stress of the antioxidant system in maize seedling roots with different drought resistance. Acta Bot Boreali-Occident Sin 22:285-290.

Wang WB, Kim YH, Lee HS, Kim KY, Deng XP, Kwak SS (2009) Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. Plant Physiol Biochem 47:570-577.

Zbíral J (2000) Determination of phosphorus in calcareous soils by Mehlich 3, Mehlich 2, CAL, and Egner extractants. Commun Soil Sci Plant Anal 31:3037-3048.

Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53:247-273.

Zhu X, Song F, Liu S (2011) Arbuscular mycorrhiza impacts on drought stress of maize plants by lipid peroxidation, proline content and activity of antioxidant system. J Food Agric Environ 9(2):583-587.