Pak. J. Bot., 51(3): 799-807, 2019. DOI: 10.30848/PJB2019-3(1)

RESPONSE OF MAIZE TO FIELD DROUGHT STRESS: OXIDATIVE DEFENSE SYSTEM, OSMOLYTES' ACCUMULATION AND PHOTOSYNTHETIC PIGMENTS

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Abstract

The study was performed to determine as to whether antioxidant potential (enzymatic and non-enzymatic antioxidants) as well as some other attributes could be used as potential drought resistance markers in two maize lines (B_{73} and MO_{17}). Under water deficit conditions, growth traits, relative water content (RWC), chlorophyll *a*, total chlorophyll and carotenoids as well as grain yield decreased significantly in both lines compared to those under control conditions. In contrast, water deficit caused a significant increase in the activities of superoxide dismutase (SOD) and catalase (CAT) isozymes as well as levels of glycine betaine (GB), proline and malondialdehyde (MDA), whereas total phenolics, total soluble protein, H_2O_2, chlorophyll *b*, chlorophyll *a*/*b* ratio, anthocyanin and peroxidase (POX) isozyme activity remained unaffected in both maize lines. Although water deficit stress induced oxidative stress in both maize lines, the enzymatic and non-enzymatic antioxidants and key organic osmolytes increased significantly in both maize lines. For example, SOD isozyme activities, and GB and proline contents were considerably greater in relatively drought resistant MO_{17} than those in relatively drought sensitive B_{73}. However, in contrast, CAT activity was higher in B_{73}. Overall, SOD isozyme activities, and GB and proline contents were found to be potential biochemical indicators of drought resistance in the two maize lines used in the present study.

Key words: Antioxidant, Chlorophyll, Glycine betaine, Maize, Proline, Water deficit.

Introduction

Drought stress can pose adverse effects on growth, development and yield output of several crops, and it is known to be the most damaging to crops compared to other abiotic stresses (Ashraf, 2010; Hao *et al.*, 2011). However, plants employ a myriad of strategies thrive under water deficit conditions. For example, under drought stress, most plants respond by synthesizing/ accumulating a variety of organic osmolytes. Of various organic osmolytes, proline and GB commonly accumulate in plants subjected to drought stress as well as other stresses. However, their definite roles in plant stress resistance remain debatable, but both have been considered beneficial for maintaining the stability of enzymes and membranes under stress conditions (Ashraf & Foolad, 2007). However, many reports have shown a considerable interaction between GB and proline accumulation and crop stress resistance (Subbarao *et al.*, 2001; Hsu *et al.*, 2003).

Water deficit stress also poses a considerable adverse effect on plant photosynthesis because in most plants the contents of key photosynthetic pigments decrease markedly thereby resulting in reduced rate of CO_2 assimilation (Cielniak *et al.*, 2006; Cruz de Carvalho, 2008; Gill & Tuteja, 2010; Zlatev & Lidon, 2012; Ashraf & Harris, 2013). Furthermore, drought stress generates high accumulation of reactive oxygen species which may damage the biological membranes (Ashraf, 2010). For example, different types of cellular ROS [superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^\bullet O_2$), and the hydroxyl radical (OH)] are frequently generated in cells under drought stress (Ashraf, 2010). All these oxidants can perturb a variety of biochemical reactions by disrupting a number of metabolites (Mittler, 2002). However, to counteract the excess production/ accumulation of ROS, most plants have the ability to generate antioxidants, enzymatic and non-enzymatic. The most promising enzymatic antioxidants are peroxidase (POX), superoxide dismutase (SOD), and catalase (CAT). Some of the key non-enzymatic antioxidants include carotenoids, polyphenols, glutathione, ascorbate, tocopherols, anthocyanins, etc. which can easily scavenge $^\bullet O_2$ and $^\bullet OH$.

It is believed that lines/cultivars differing in stress tolerance can synthesize/accumulate the antioxidants differently. For example, Ashraf (2010) reported that mlondialdehyde (MDA) and H_2O_2 contents were higher in the leaves of a sensitive genotype of maize than those in tolerant ones under drought stress. Many reports indicate that drought stress induces MDA content in leaves and roots (Cruz de Carvalho, 2008). Drought stress enhanced the MDA and H_2O_2 contents in leaves of maize lines (Chugh *et al.*, 2011). An increase in content of MDA under drought stress has been reported in leaves of thought sensitive maize line at seedling stage, whereas no change is observed intolerant lines (Azooz *et al.*, 2009). In view of a number of reports antioxidant response is correlated with tolerance of individual crop cultivars to abiotic stresses including drought stress (Athar *et al.*, 2008; Kolarovic *et al.*, 2009). For example, dehydration during pre- and post-flowering stages of maize crop increased the activity of various antioxidant enzymes (Azooz *et al.*, 2009). In another study, a positive correlation between maize oil qualities, phenolic contents and carotenoid levels have been observed under water deficit conditions (Ali *et al.*, 2010). The present study was performed with a hypothesis that water deficit-induced change in antioxidant system could affect yield and growth of maize plants grown under drought conditions. Therefore, the present study was performed to evaluate the effect of water deficit stress on plant growth, plant height and grain yield of maize. In addition, it was examined as to whether RWC, photosynthetic pigments, GB and proline contents, enzymatic activities (SOD, POX and CAT) and non-enzymatic antioxidant contents in two well-known lines of maize (B_{73} and MO_{17}) were influenced due to drought stress and which of them could be used as indicators of drought tolerance in maize.

Materials and Methods

Plant materials and field experiments: A field trial was carried out, during two appropriate growing seasons (2014-2015) at the Research Station of University of Tabriz. The experiment was performed in a randomized complete block design with two factors: two maize inbred lines (B_{73} and MO_{17}), and two water deficit conditions (one with commonly available irrigation water referred to as control and the other interrupted irrigation during 27 days before flowering). The maize seeds were planted on May 3, 2014, and April 25, 2015. The experimental plots included two rows of 3 m long and 0.75 m wide. The soil at the experimental site was sandy-loam (sand 49.8%, clay 18.5%). The soil properties were determined before planting following Carter & Gregorich (2008) and presented in Table 1. The fertilization was done 25 days after planting by adding 60 kg ha^{-1} urea. The grain yield per plant, plant height, plant dry weight, number of leaves and stem diameter were evaluated.

Physiological traits: The leaf water content (RWC) was estimated following the gravimetric method as described by Chen *et al.,* (2012). The pigments such as anthocyanins, chlorophyll *a, b* and carotenoids were analyzed using 200 mg of fresh leaf sample in a pestle and mortar containing liquid N, and at the powder stage, precooled extraction solvent [2.0 ml acetone (85%) and Tris stock buffer (15%), pH 8.0] were added. The mixture was centrifuged at 12,000 *g* for 3 min and the optical density of the supernatants was measured at 537, 663, 647 and 470 nm using a spectrophotometer (Yaryura *et al.,* 2009).

Biochemical traits: Glycine betaine was determined using the protocol described by Grieve & Grattan (1983). To a sample of 500 mg fresh leaves, 5 ml otoluene (0.05%) were added. All tubes containing the leaf samples were shaken on an electrical shaker at 25°C for one day. Then an aliquot (1 ml) after filtration was mixed with 1 ml of 2 *N* HCl, thereafter 0.01 ml of KI_3 solution was added and vortexed in an ice cold water bath for one hour. An ice-cooled water (2 ml) and 10 ml of chilled 1, 2 dichloroethane were added to each tube. Two layers of the mixture appeared when an air stream was passed for 1-2 min. The OD of the lower layer (organic) was recorded at 365 nm.

Proline content of fresh leaves was measured using the procedure described by Bates *et al.,* (1973). Samples of the leaf (each 500 mg) were ground in 3% (w/v) sulfosalicylic acid, and centrifuged (4000 *g*) for 10 min at 4°C. The supernatant so resulted was treated with glacial acetic acid and acid ninhydrin. By treating the mixture with toluene the ODs of all samples were recorded at 520 nm.

The levels of hydrogen peroxide (H_2O_2) were recorded following the procedure reported by Velikova *et al.,* (2000) using 0.1% (w/v) TCA, 10 mM potassium phosphate (pH 7.0) buffer and 1 ml (1 M) KI. The optical density of the supernatant was noted at 390 nm. The level of leaf lipid peroxidation was measured following Cakmak & Horst (1991). For determining total phenolics, leaf tissue (0.05 g) was ground well in 80% acetone and subjected to centrifugation at 10,000 *g* for 10 min. To an aliquot of 100 µl of the supernatant, 2 ml of water and 1 ml of Folin–Ciocalteau's phenol reagent were added and shaken vigorously. Thereafter, 5 ml of 20% sodium carbonate solution were added and the volume was raised to 10 ml by adding distilled water. The absorbance of all treated samples was read at 750 nm (Noreen & Ashraf, 2009).

Antioxidant enzymes and electrophoresis: The crude extracts of fresh leaves were prepared in a tris-HCl extraction buffer (Tris 50 mM, pH 7.5, ascorbic acid (50 mM), sucrose 5%, sodium metabisulfite (20 mM), PEG (2%) and 2- mercaptoethanol (0.1%) before use (Valizadeh *et al.,* 2013), with a ratio of 0.5 mg μl^{-1} (1W: 2V) and centrifuged at 4°C and 10,000 *g* for 10 min using small Eppendorf tubes. The enzyme extracts were immediately absorbed on a filter paper and loaded onto 7.5% horizontal slab polyacrylamide gels (0.6×15×12 cm) using the TBE (Tris-Borate-EDTA) electrode buffer (pH = 8.8). Electrophoresis was carried out at 4°C for 3 h. Three enzymatic systems were analyzed in this study. Two slices prepared from the slab gels were stained after electrophoresis for enzyme activities. The staining protocol for superoxide dismutase (SOD) was performed according to Soltis & Soltis (1990), peroxidase (POX) and catalase (CAT) according to Olson & Varner (1993). The stained gels were fixed and scanned at suitable state. The protein content of the enzyme extracts was determined following Bradford (1976). An image analysis "MCID software" was used to measure optical density × area for each isozyme activity.

Statistical analysis

The trial was conducted in a randomized complete block design with two factor factorial arrangement and three replicates. Analysis of variance and significant differences among the treatment means were calculated by the least-significant-difference (LSD) test at $p<0.05$ level.

Table 1. Chemical properties of the soil used.

pH	CCE (%)	OM (%)	Na (mg kg^{-1})	K (mg kg^{-1})	P (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
7.7	9.8	1.3	195	836	27	3.3	9.9	1.7	1

CCE: Calcium carbonate equivalent, OM: Organic matter

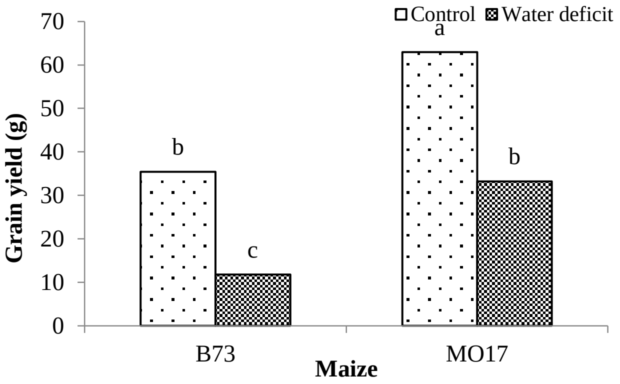

Fig. 1. Effects of water deficit on the grain yield per plant of maize lines. The data are the averages of values observed during the two field experiments in 2014 and 2015. Means with the same letter are not significantly different by the least significant difference (LSD) at $p<0.05$.

Results

Water deficit imposed in this study led to changes in grain yield and growth traits in the two maize lines (Figs. 1 and 2, and Table 2). The average grain yields over the two years were 35.4 and 62.9 g plant^{-1} for B_{73} and MO_{17}, respectively, under control irrigation. Water deficit stress caused a significant decrease in grain yield in both lines. This decrease was 67% and 47% for B_{73} and MO_{17}, respectively. Thus, MO_{17} could be considered as "drought resistant" and B_{73} as "drought sensitive" to water deficit (Fig. 1). Plant height was also reduced markedly in both maize lines under water deficit conditions (Fig. 2). This decrease was 46% for B_{73} and 40% for MO_{17}. Plant dry weight was also markedly decreased in both maize lines under water deficit conditions (Fig. 2). This decrease was 25% for B_{73} and 33% for MO_{17}.

Water deficit stress reduced RWC in both inbred lines when compared with the control conditions (Fig. 3). The reduction in leaf RWC was higher in B_{73} (27%) than that in MO_{17} (14.5%). Leaf pigment contents from both control and stress conditions of the two maize lines are presented in Table 3. The B_{73} and MO_{17} lines did not show significant differences in pigment content under control conditions except for carotenoid content. Water deficit stress caused a marked reduction in chlorophyll *a*, total chlorophyll and carotenoids in both maize lines, but this decrease was significantly higher in MO_{17} than that in B_{73}. These reductions were approximately 4, 22 and 23 percent for carotenoids, total chlorophyll and chlorophyll *a*, respectively. The leaf chlorophyll *b*, chlorophyll *a*/*b* ratio and anthocyanins in both maize lines were not significantly changed under water deficit stress compared to those control conditions.

Glycine betaine (GB) and proline concentrations in the two maize lines were increased during water deficit (Table 4). The concentrations of GB and proline were significantly higher in line MO_{17} than those in B_{73}. Total soluble protein content was not significantly changed in both lines under water stress (Table 4). Drought stress had an opposite effect on total phenolics in both maize lines. The total phenolic content was increased in B_{73} (3.6%), whereas it was decreased in MO_{17} (6%). H_2O_2 content in both maize lines was not significantly changed under water deficit stress compared to that under control conditions (Table 4). MDA content was increased in both maize lines under drought stress, but it was much higher in B_{73} than that in the other line (Table 4).

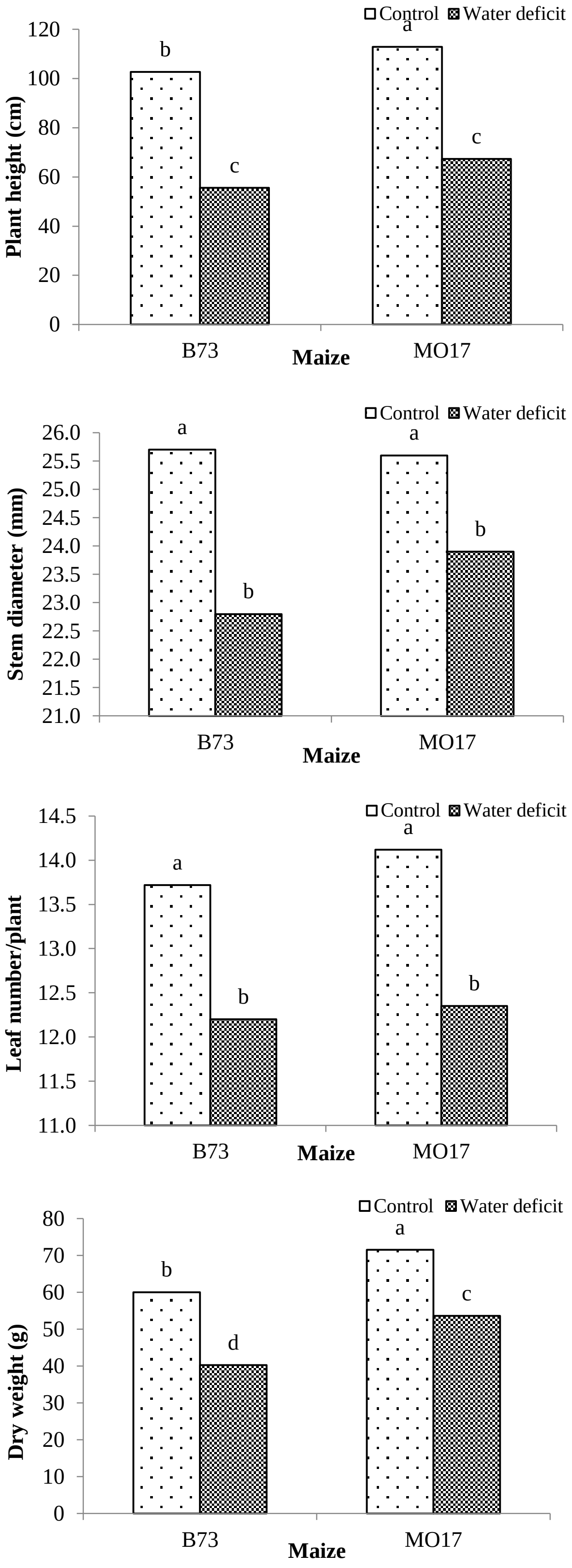

Fig. 2. Effects of water deficit on the plant height, stem diameter, leaf number and dry weight of two maize lines (B_{73} and MO_{17}). The data are the averages of values observed during the two field experiments in 2014 and 2015. Means with the same letters are not significantly different by the least significant difference (LSD) at $p<0.05$.

Table 2. *P*-values of ANOVA analysis of agronomical, physiological and biochemical triats of maize inbred lines under field condition over two years.

Triat	Main-factor effects		Interaction effects
	M	W	M × W
Grain yield	<0.0001	<0.0001	<0.001
Plant height	<0.045	<0.0001	<0.001
Stem diameter	<0.825	<0.720	<0.842
Leaves number	<0.315	<0.0002	<0.565
Dry weight	<0.0001	<0.0001	<0.0001
RWC	<0.425	<0.032	<0.021
Chlorophyll *a*	<0.042	<0.019	<0.001
Chlorophyll *b*	<0.521	<0.190	<0.215
Total chlorophyll	<0.0001	<0.032	<0.222
Chlorophyll *a*/*b* ratio	<0.521	<0.028	<0.325
Anthocyanin	<0.610	<0.520	<0.347
Carotenoid	<0.0001	<0.0001	<0.001
Total phenolics	<0.702	<0.032	<0.692
Total soluble protein	<0.752	<0.285	<0.514
Glycine betaine	<0.0001	<0.0001	<0.001
Proline	<0.0001	<0.0001	<0.001
MDA	<0.0001	<0.0001	<0.001
H_2O_2	<0.525	<0.500	<0.625
SOD_1	<0.0001	<0.0001	<0.0001
SOD_2	<0.0001	<0.0001	<0.0001
SOD_3	<0.0001	<0.0001	<0.0001
POX_1	<0.0001	<0.052	<0.100
POX_2	<0.041	<0.219	<0.321
POX_3	<0.035	<0.056	<0.102
CAT	<0.0001	<0.0001	<0.0001

'M': Effect of maize inbred lines; 'W': Effect of water deficit conditions; M × W: Interaction effects

Table 3. Effects of water deficit stress on pigment contents (±S.E; n=6) in B_{73} and MO_{17} lines under field condition over two years.

Pigment contents (µmol/g fresh weight)	Maize line			
	B_{73}		MO_{17}	
	Control	Water deficit	Control	Water deficit
Chlorophyll *a*	0.019 ± 0.001^a	0.015 ± 0.001^b	0.018 ± 0.002^a	0.010 ± 0.003^c
Chlorophyll *b*	0.005 ± 0.001^a	0.005 ± 0.002^a	0.005 ± 0.001^a	0.004 ± 0.001^a
Total chlorophyll	0.024 ± 0.001^a	0.020 ± 0.002^b	0.023 ± 0.001^a	0.014 ± 0.002^c
Chlorophyll *a*/*b* ratio	3.800 ± 0.828^a	3.001 ± 1.025^a	3.600 ± 1.420^a	2.50 ± 1.025^a
Anthocyanin	0.064 ± 0.025^a	0.058 ± 0.020^a	0.066 ± 0.022^a	0.057 ± 0.022^a
Carotenoid	0.011 ± 0.001^a	0.009 ± 0.001^b	0.009 ± 0.001^b	0.007 ± 0.001^c

Means with the same small letters were not significantly different from the least significant difference (LSD) at $p<0.05$.

Table 4. Antioxidants and other biochemical stress markers (±S.E; n=6) in two maize lines (B_{73} and MO_{17}) under field in control and water deficit conditions.

Trait	Maize line			
	B_{73}		MO_{17}	
	Control	Water deficit	Control	Water deficit
Total phenolics (mg/g fresh weight)	2.327 ± 0.206^a	2.411 ± 0.304^a	2.471 ± 0.145^a	2.326 ± 0.322^a
Total soluble protein (mg/g fresh weight)	0.938 ± 0.038^a	0.939 ± 0.046^a	0.939 ± 0.047^a	0.870 ± 0.010^a
Glycine betaine (µmol/g dry weight)	0.666 ± 0.010^d	0.761 ± 0.016^b	0.700 ± 0.017^c	0.854 ± 0.023^a
Proline (µmol/g fresh weight)	3.008 ± 0.002^d	5.145 ± 0.003^b	3.460 ± 0.001^c	6.157 ± 0.004^a
MDA (nmol/g fresh weight)	20.019 ± 0.006^b	20.211 ± 0.036^a	15.154 ± 0.006^c	18.088 ± 0.013^b
H_2O_2 (µmol/g fresh weight)	1.949 ± 0.020^a	0.955 ± 0.009^a	1.984 ± 0.017^a	1.955 ± 0.007^a

Malondialdehyde (MDA); Hydrogen peroxide (H_2O_2). Means with the same small letters were not significantly different from the least significant difference (LSD) at $p<0.05$

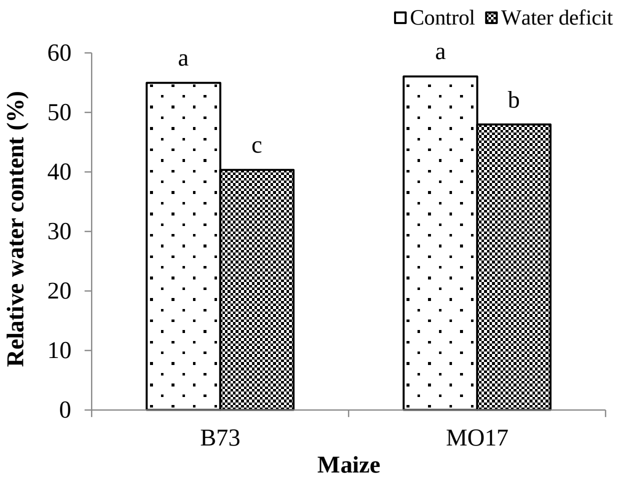

Fig. 3. Leaf relative water content (RWC) in both maize lines under control and water deficit conditions. The data are the averages of values during the two field experiments in 2014 and 2015. Means with the same letter are not significantly different by the least significant difference (LSD) at $p<0.05$.

Appraisal of antioxidant enzyme activities in the leaves of two maize lines under control and water deficit conditions was based on staining onto the same gels, which showed a significant increase in SOD and CAT antioxidant isoforms in water deficit condition compared to those under control. But this was not true for the POX isozymes (Figs. 4 and 5). For SOD, POX and CAT, three, two and one isoforms were observed, respectively (Fig. 4 A, B and C). The antioxidant activity of the three SOD isozymes was exhibited mainly by SOD_2 in MO_{17} maize line under water stress (Fig. 5). Water deficit stress induced changes in POX_1 and POX_3 were non-significant, but POX_2 increased to a slight extent in MO_{17}, but no such significant change was observed in B_{73} line compared with that under control conditions (Fig. 5). Water deficit stress significantly increased the CAT activity in both maize lines. The CAT activity was higher in B_{73} than that in line MO_{17} under water deficit stress (Fig. 5). The applied water deficit stress enhanced SOD and CAT activities about 32 and 76 percent, respectively.

A multiple regression analysis on grain yield of maize lines was employed under water stress conditions. This analysis showed that grain yield reduction in different maize inbred lines was interrelated with SOD isozyme activities, and GB and proline contents (Table 5).

Table 5. Multiple regression analysis showing the dependence of grain yield in per plant on plant growth, relative water content (RWC), pigment contents and biochemical attributes of both maize inbred lines under water deficit in field conditions (R^2= 0.89).

Variable	Coefficient
Intercept	-0.020^{ns}
Plant height	0.050^{ns}
Stem diameter	0.112^{ns}
Leaves number	0.082^{ns}
Dry weight	0.211^{ns}
RWC	-0.046^{ns}
Chlorophyll *a*	-0.009^{ns}
Chlorophyll *b*	-0.005^{ns}
Total chlorophyll	-0.011^{ns}
Chlorophyll *a*/*b* ratio	0.015^{ns}
Anthocyanin	-0.005^{ns}
Carotenoid	-0.006^{ns}
Total phenolics	0.012^{ns}
Total soluble protein	-0.018^{ns}
Glycine betaine	0.445*
Proline	0.609*
MDA	0.005^{ns}
H_2O_2	0.030^{ns}
SOD_1	0.653*
SOD_2	0.738*
SOD_3	9.893**
POX_1	-0.032^{ns}
POX_2	-0.037^{ns}
POX_3	-0.106^{ns}
CAT	-0.080^{ns}

Relative water content (RWC); Malondialdehyde (MDA); Hydrogen peroxide (H_2O_2); Isoforms of superoxide dismutase (SODs); Isoforms of peroxidase (POXs); Catalase (CAT)

ns, *, **: non-significant and significant differences at 5 and 1% probability, respectively

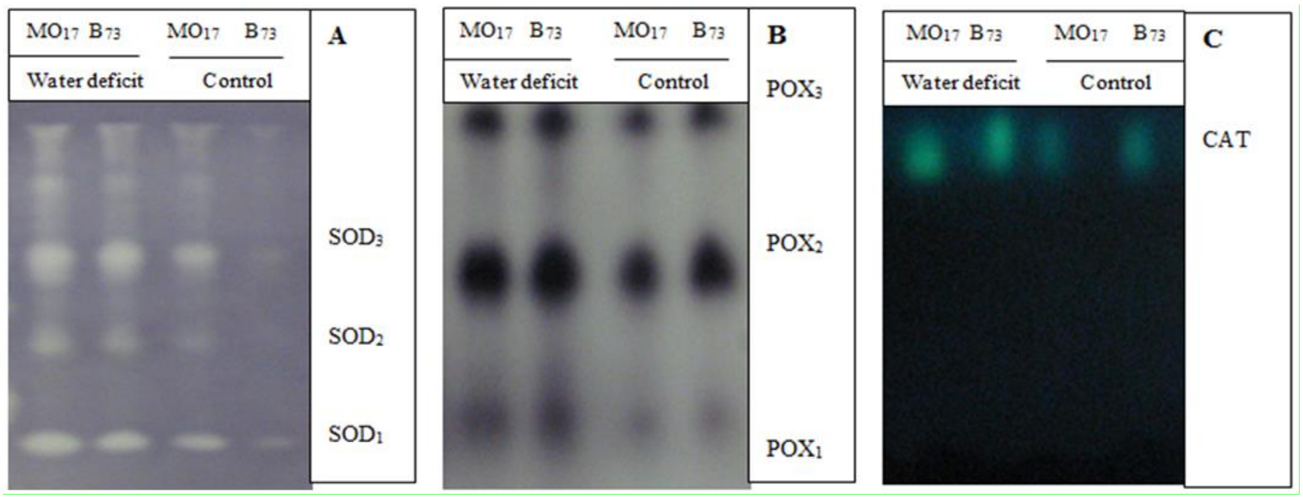

Fig. 4. (A) Banding pattern of superoxide dismutase (SOD), (B) peroxidase (POX), and (C) catalase (CAT) in control and water deficit stress under field conditions in B_{73} and MO_{17} lines.

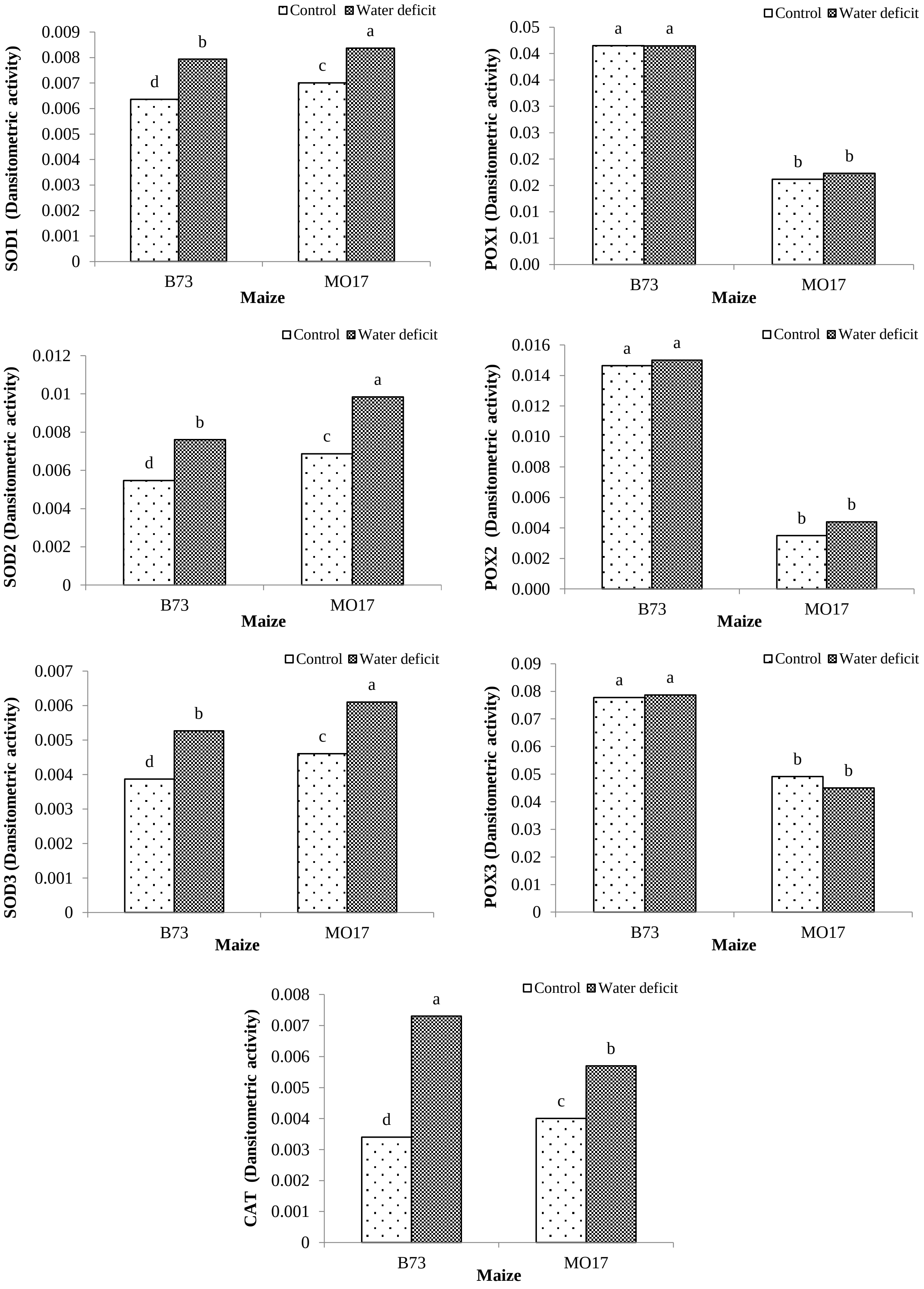

Fig. 5. Mean activities of superoxide dismutase (SODs), peroxidase (POXs), and catalase (CAT) isoforms of B_{73} and MO_{17}lines under filed control and water deficit conditions. Means with the same letter are not significantly different from the least significant difference (LSD) at $p<0.05$.

Discussion

Percent inhibition in grain yield under water stress indicated that line MO_{17} was relatively ranked as “drought resistant” and B_{73} as “drought sensitive” to water deficit (Fig. 1A). Reduction in grain yield and plant growth in the two maize liens caused by water stress could be attributed to some key physio-biochemical processes regulating plant growth (Chugh *et al*., 2011), e.g., change in photosynthesis, nutrient uptake/accumulation, osmolyte accumulation, enzyme activities, etc. Changes in all these processes are believed to affect crop growth (Anjum *et al*., 2011; Moharramnejad *et al*., 2015). In some other studies with maize, drought stress has been found to be detrimental for crop growth and yield. For example, Campos *et al*., (2006) found 45-60% yield reduction in maize when drought was imposed at the silk emergence stage. In a similar study, Cakir (2004) reported a marked decrease in grain yield and plant height in maize when it was subjected to short-term drought. In another study on maize plants, water deficit conditions significantly disrupted plant height, leaf number plant^{-1}, total leaf area plant^{-1}, shoot dry weight plant^{-1}, root dry weight plant^{-1}, number of grains plant^{-1}, and grain yield plant^{-1} (Talaat *et al*., 2015).

Plants, for their survival under water deficit conditions, tend to retain a reasonable amount of water so as to ensure the normal functioning of all cellular metabolic processes. It is generally believed that RWC decreases in most plants in response to water deficit stress (Shaw *et al*., 2002; Bürling *et al*., 2013). Our results also show that, RWC was decreased in the two maize lines in response to drought stress, although the decrease in RWC was much slower in line MO_{17} than that in B_{73} (Fig. 3). These observations are similar to those of Yan *et al*., (2016).

Water shortage is known to inhibit photosynthesis by damaging the photosynthetic apparatus (Nayyar & Gupta 2006). In this experiment, water stress caused significant decrease in chlorophyll content in both maize lines. Analogous to these findings, in an earlier study, reduction in chlorophyll pigments was reported in maize plants subjected to progressive drought stress (Anjum *et al*., 2011). Plant biomass production was affected directly by the chlorophyll pigments’ reduction in stressed plants. Dcrease in photosynthetic pigments may lead to less utilization of energy and carbon demand for chlorophyll synthesis (Zhang *et al*., 2012; Zlatev & Lidon, 2012). Some pigments can be function as accessory pigments in photosynthesis, although their concentration could also be declined as a part of plant response (Silva *et al*., 2010; Zhang *et al*., 2012; Moharramnejad *et al*., 2015).

Among various secondary metabolites, soluble phenolics are believed that they have an important role in plants exposed to abiotic stresses (Ruiz & Romero, 2001). For example, increased synthesis/accumulation of soluble phenolics was reported to be importantly correlated with the heat and salt tolerance in sugarcane (Wahid & Ghazanfar, 2006). In contrast to our study, water deficit stress caused a higher decrease in total phenolics in the drought resistant maize line MO_{17} than that in the drought sensitive one, showing a negative relationship between drought resistance and accumulation of total phenolics in maize plants. Leaf phenolic compounds on the R_5 growth of peanut genotypes grown at field capacity or terminal drought were not significantly different at different stages in two years (Aninbon *et al*., 2016). In another study, drought reduced polyphenols in all cotton genotypes irrespective of their degree of drought resistance (Yildiz-Aktas *et al*., 2009; Ali *et al*., 2010).

Glycine betaine (GB) and proline concentrations in stressed plants can resist membrane integrity, may decrease lipid membrane oxidation, protection and stabilize ROS scavenging enzymes, and take part in stabilizing sub-cellular structures, scavenging free radicals, and buffering cellular redox potential (Tuna *et al*., 2007; Ashraf & Foolad, 2007). In the present study, accumulation of GB and proline occurred in both maize lines exposed to water deficit conditions. These osmolytes are believed to play a vital role in cellular osmotic adjustment (Kavi Kishore *et al*., 2005; Moharramnejad *et al*., 2015; Talaat *et al*., 2015). In our study, higher levels of GB and proline in both lines might have been involved in osmoregulation, which in turn may allow additional water to be taken up by the maize plants from the growth medium thereby counteracting the adverse effects of drought stress on plant metabolism (Kumar *et al*., 2003). Water deficit induced, increase in GB and proline accumulations in maize plants and might be a part of a mechanism which prevents water loss in plants through osmotic adjustment (Talaat *et al*., 2015).

In our study, lipid peroxidation measured as the amount of MDA in maize leaves differed between the two lines. However, under water deficit stress in line MO_{17}, MDA content was lower than that in B_{73} (Table 4). In another study with maize, an increase was observed in MDA content in a drought sensitive genotype at the seedlings stage, whereas no change was observed in a tolerant genotype (Moussa & Abdel-Aziz 2008; Moharramnejad *et al*., 2015). Furthermore, MDA concentration was increased significantly in maize plants under water deficit stress, and was well correlated with H_2O_2 levels (Talaat *et al*., 2015).

Increased SOD and CAT activities may efficiently scavenge harmful ROS that was clearly indicated by the decreased O_2, H_2O_2, and MDA contents in maize plants (Anjum *et al*., 2011; Talaat *et al*., 2015). Plants have the ability to detoxify ROS by up-regulating antioxidant enzymes, like SOD, POX and CAT. Malan *et al*., (1990) reported a higher level of SOD in drought tolerant maize inbreds compared to that in drought sensitive ones. Kolarovic *et al*., (2009) have reported enhanced activity of POX in drought tolerant as well as sensitive maize seedling exposed to treated osmotic stress. Numerous studies have reported that higher levels of antioxidants are associated with plant drought tolerance (Ashraf, 2010; Valizadeh *et al*., 2013; Moharramnejad *et al*., 2016).

Taken overall, SOD isozyme activities and GB and proline contents were found to be potential biochemical indicators of drought resistance in two maize lines examined in the present investigation.

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(Received for publication 15 February 2018)