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# Banding Patterns Activity of Antioxidant Enzymes and Physiological Attributes in Maize (*Zea mays* L.) Families under Water Deficit Stress

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## ABSTRACT

**In order to assess the effect of water-deficit stress on the activity of antioxidant enzymes and agro-physiological attributes of maize, a split-plot field experiment was conducted with three replications based on the randomized complete block design. Three levels of irrigation (well-watered, intermediate and severe water-deficit stress) and four maize families including 10 genotypes were considered as the main and sub factors, respectively. Post treatment, the electrophoretic analysis of three enzymes in maize leaves including SuperOxide Dismutase (SOD), Catalase (CAT) and Peroxidase (POX) was carried out on 8% horizontal acrylamide gel. Moreover, agro-physiological attributes such as MalonDiAldehyde (MDA), $H_2O_2$, chlorophyll index (SPAD), Relative Water Content (RWC), and grain yield were measured. Concomitantly with increasing intensity of water-deficit stress, the activity of most isozymes and the contents of MDA and $H_2O_2$ increased while POX2 activity, chlorophyll index, *RWC*, and grain yield decreased. Under intermediate stress, POX1 isozyme in Lia0688 line (233%) and, under severe stress, POX2 isozyme in AR68 hybrid (201%) showed higher increase compared with the well-watered treatment. Overall, POX1, SOD2, CAT isozymes and MDA, chlorophyll index, and *RWC* were identified as suitable traits. Based on enzyme activity and agro-physiological attributes, SC706 and TWC647 hybrids were superior to the other genotypes and expressed higher tolerance to water deficit stress. Moreover, among parental lines, MO17, B73 and Lia0688 were promising, although Lia0688 and MO17 were more tolerant lines and showed better performance compared with the line B73 and other lines under well-watered and stress conditions.**

**Keywords:** Drought tolerance, Irrigation, Isozymes, Oxidative stress, Selection index.

## INTRODUCTION

Maize (*Zea mays* L.) is a cereal plant with a relatively short growing period and high performance, and takes the third position after wheat and rice (Xu *et al*., 2004). Well adapted to different climatic conditions, it is one of the most important crops in the temperate and semitropical areas and is currently grown in many countries. It is a multidisciplinary crop used as human food, animal feed, fodder and biofuel (FAOSTAT 2013). Iran is a semi-arid country and produces about 1.2 million tons of maize per year, which is equivalent to 1.52% of its total crop production and 6.41% of the total cereal production. Here, approximately 166,000 ha are under maize, which is equivalent to 1.46% of the total cropped area and 2.03% of the total area of cereal harvest (Iran Agricultural Products Statistics, 2015; FAOSTAT, 2015).

More than 45% of the world's agricultural lands, in which 38% of the world's population live, are exposed to drought or drought stress (Ashraf and Foolad, 2007). Water deficiency occurs when soil water potentials are sufficiently negative to reduce water availability to sub-optimal levels for plant

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growth and development (Boyer, 1982). Plants have evolved several different types of drought-adaptive strategies that allow them to adapt to specific habitats for the benefit of their growth and development (Fang and Xiong, 2015). The plants tolerant to water deficit react to the environmental stresses by making changes in some of their physiological characteristics, including the increase in the concentration of components with a small molecular weight (proline, glucose, betaine, etc.) that are generally called compatible solutions (Bajji *et al*., 2001). Water deficit induces Reactive Oxygen Species (ROS) contents. In this condition, the activity of antioxidant enzymes increases (Hayat and Ahmad, 2007). Effect of water deficit stress depends on the growth stage, severity and the duration of stress, plant species, and the stage of plant growth (Cruz de Carvalho, 2008). Understanding plant response to water deficit is very important and forms the main part of the structure of drought tolerant plants (Zhao *et al*., 2008; Chaves *et al*., 2009). Khanna-Chopra and Selote (2007) reported that under intermediate drought stress at seedling stage, the drought-tolerant genotypes showed better ability to adapt and acclimatize to drought stress compared with the susceptible ones by maintaining optimum water relations and less damage to membrane because of the lower concentration of $H_2O_2$ and antioxidant defense system in the leaves under water-deficit stress condition.

Produced ROSs under water deficit stress can destroy proteins, lipids, carbohydrates, and nucleic acids (Bian and Jiang, 2009). In order to decrease the oxidative effects of ROSs, plants have developed various mechanisms (Cruz de Carvalho, 2008). Enzymatic and non-enzymatic antioxidant systems are one of the mechanisms that scavenge ROS. In the antioxidant defense system, the enzymes of SuperOxide Dismutase (SOD), Peroxidase (POX), Catalase (CAT), Ascorbate Peroxidase (APX), and Glutathione Reductase (GR) are considered the key enzymes and the most important non-enzymatic antioxidant compounds are glutathione, alpha-tocopherol, and ascorbic acid (Salin, 1991). The CAT directly causes the decomposition of hydrogen peroxide and the POX decomposes hydrogen peroxide by the use of phenolic compounds of electron donor, while the SOD produces hydrogen peroxide by dismutating superoxide anions (Gaber, 2010). Changes in the enzymatic activities under water stress are well documented. Various studies indicated that the activity of antioxidant enzymes has positive correlation with the plant's tolerance to abiotic stress such as reaction to the drought stress in wheat (Shao *et al*., 2007; Abdullah and Ghamdi, 2009; Hasheminasab *et al*., 2012) and in maize (Moharramnejad *et al*., 2016). Therefore, the activity of antioxidant enzymes could be used as selection index for selecting stress tolerant genotypes. In any case, contradictory results (increase, decrease or remaining unchanged) in most studies have been obtained by the analysis of antioxidant enzymes in different conditions (Badiani *et al*., 1990; Sharma and Dubey, 2005; Simov-Soilova *et al*., 2010; Naderi *et al*., 2014).

The aim of this study was to complete the information concerning activity behaviors of physiological attributes and antioxidant isozymes of SOD, CAT, and POX in four maize families under field water-deficit stress conditions.

## MATERIALS AND METHODS

### Plant Materials and Experimental Design

The plant materials included four common and commercial single cross hybrids and their 8 parent lines (two of them were common in different families) (Table1). A field split plot experiment was carried out based on a randomized complete block design with three replications. Each genotype was cultivated in 3 rows with 3 m long, 70 cm apart. The main factor in this study was water deficit at three levels including: (i) Well-Watered (WW), with irrigation every 7 days in summer; (ii) Intermediate or post-flowering Stress (IS), where irrigations was suspended 1 to 2 weeks after-flowering; and (iii) Severe or pre- and post-flowering Stress (SS), where irrigations was suspended 1 to 2 weeks prior to anthesis until 1 to 2 weeks after-flowering, (Bolanos





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**Table1.** Number, codes and sources of maize genotypes**.**

| Num. | Genotypes | Paternal line | Maternal line | Seed-providing center |
|---|---|---|---|---|
| 1 | MO17 (Line) | --- | --- | MARC [a] |
| 2 | B73 (Line) | --- | --- | MARC [a] |
| 3 | AR68(F1) | Sa0689 | Lia0688 | SPPII [b] |
| 4 | TWC647(F1) | MO17 | SC647 | MARC [a] |
| 5 | SC706(F1) | MO17 | K3547/4 | MARC [a] |
| 6 | Sa0689 (Line) | --- | --- | SPPII [b] |
| 7 | K1264/1 (Line) | --- | --- | SPPII [b] |
| 8 | SC647(F1) | K1264/1 | B73 | MARC [a] |
| 9 | K3547/4 (Line) | --- | --- | MARC [a] |
| 10 | Lia0688 (Line) | ---- | ---- | SPII [b] |

[a] Moghan Agricultural Research Center. [b] Seed and Plant Improvement Institute.

and Edmeades, 1996). In this study, 10 maize genotypes were arranged in sub-plots.

### Physiological Attributes:

For physiological attributes, a randomly selected sample from flag leaves was taken. The attributes measured were as follows:

Hydrogen peroxide ($H_2O_2$): Hydrogen peroxide levels were determined according to Velikova *et al*. (2000).

MalonDiAldehyde (MDA): The level of lipid peroxidation was determined in terms of ThioBarbituric Acid-Reactive Substances (TBARS) concentration as described by Carmak and Horst (1991).

Chlorophyll index: Leaf chlorophyll content was measured by SPAD (502 Plus Chlorophyll Meter) chlorophyll meter 24 hours after applying water stress.

Relative Water Content (RWC): Relative water content (RWC) of leaves was calculated using the following equation:

RWC= *(Fresh weight – Dry weight)/ (Turgid weight – Dry weight) ×100*

Total soluble proteins: The concentration of the total soluble proteins was determined by Bradford (1976) method.

Native electrophoresis: Slab Polyacrylamide gels (0.6×15×12 cm) were prepared using Poulik buffer (Poulik, 1957) with 8% gel concentration. Enzymes extraction was performed according to Valizadeh *et al.* (2013) by using fresh and green leaves collected from three random plant in each sub-plot with a ratio of 1:1 (W: V) The homogenate was centrifuged (Model EBA 12R) at 4ºC and 10,000 rpm for 10 minutes. Supernatants were immediately absorbed onto 3×5 mm wicks cut from Whatman 3 mm filter paper, and were loaded on to slab gels. Electrophoresis was carried out at 4℃ for 3 hours (constant current of 25 mA, and voltage of about 180V, and used TBE electrode buffer (pH 8.8)). After electrophoresis, two slices of slab gel were prepared. The staining protocol for SOD and CAT was performed according to Soltis and Soltis (1990), and for POX according to Olson and Varner (1993). The gels were fixed and scanned immediately after staining. An image analysis program (MCID Analysis Evaluation 0.7) was used to measure D×A (Optical Density×Area) parameter for each isozyme band to evaluate the enzymatic activity.

### Statistical Analysis

Data were analyzed using the general liner model procedure in SAS 9.1 program (SAS Institute, Cary, USA) based on split-plot design. The assumptions of variance analysis were tested by ensuring that the residuals were random and homogenous, with a normal distribution. Enzymatic activity was quantified by MCID software and physiological traits means were compared by Duncan test ($P \leq 0.05$).

## RESULTS

In this study, one CAT (Figure 1), two POX (POX1 and POX2) (Figure 2) and three SOD





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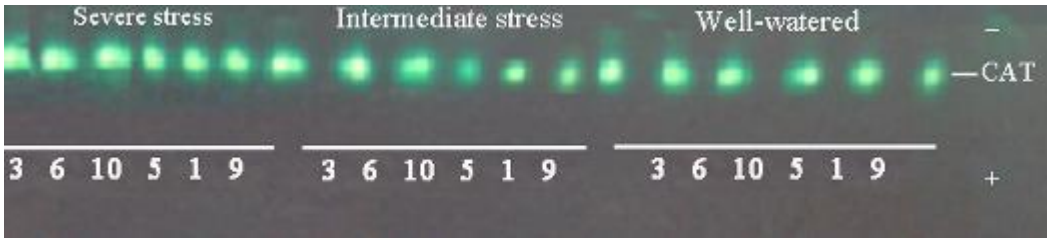


**Figure 1**. A sample of isozyme pattern and relative activity of CAT in the leaves of six maize genotypes [3 (AR68), 6 (Sa0689), 10 (Lia0688), 5 (SC706), 1 (MO17) and 9 (K3547/4)] exposed to well-watered, intermediate and severe water deficit stress.

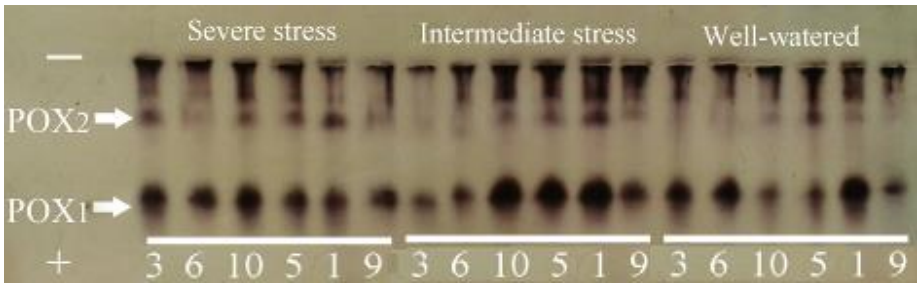


**Figure 2**. A sample of isozyme pattern and relative activity of POX in leaves of six maize genotypes [3 (AR68), 6 (Sa0689), 10 (Lia0688), 5 (SC706), 1 (MO17) and 9 (K3547/4)] exposed to well-watered, intermediate and severe water deficit stress.

(SOD1, SOD2 and SOD3) (Figure 3) isozymes were identified in the studied maize genotypes. The results of ANOVA analysis showed that there were significant differences between genotypes in terms of agro-physiological attributes and enzymatic activities, except the activity of CAT and SOD1 and the content of MDA. The effect of water deficit stress was significant on all enzymes activity, except POX2. Moreover, the interaction effect of genotype× water deficit stress on the activity of SOD2 and SOD3, leaf chlorophyll content, *RWC*, $H_2O_2$, total protein, and grain yield was significant (data not shown). The mean comparison (Table 2) showed that the activity of most isozymes was increased concomitantly with increasing stress intensity, although in that case, the activity of POX2 was not significant. In this study, the SOD2 isozyme showed the highest activity under intermediate and severe water stress conditions. Concomitantly with the increasing stress intensity, under severe stress the content of the leaf chlorophyll, *RWC* of the leaf and grain yield decreased compared with well-watered condition, while the content of MDA and $H_2O_2$ increased (Table 3). In the case of physiological attributes, MDA showed more increase with higher stress intensity (intermediate and severe stress) compared with

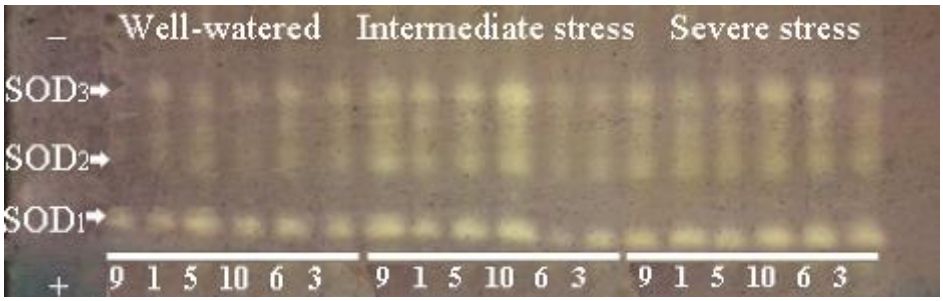


**Figure 3**. A sample of isozyme pattern and relative activity of SOD in leaves of six maize genotypes [3 (AR68), 6 (Sa0689), 10 (Lia0688), 5 (SC706), 1 (MO17) and 9 (K3547/4)] exposed to well-watered, intermediate and severe water deficit stress.





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**Table 2.** Mean and increment or decrement percentage (in parenthesis) of antioxidant enzymes activities in maize genotypes exposed to different water deficit levels.[a]

| Water deficit level | CAT | POX1 | POX2 | SOD1 | SOD2 | SOD3 |
|---|---|---|---|---|---|---|
| Well-watered | 0.0039[c] | 0.0239[b] | 0.0126[a] | 0.0073[b] | 0.0036[b] | 0.0047[c] |
| Intermediate stress | 0.0051[b] (30.8) | 0.0308[a] (28.9) | 0.0128[a] (1.6) | 0.0089[a] (21.9) | 0.0064[a] (77.8) | 0.0073[b] (55.3) |
| Severe stress | 0.0067[a] (71.8) | 0.0296[a] (23.8) | 0.0122[a] (-3.2) | 0.0099[a] (35.6) | 0.0065[a] (80.6) | 0.0085[a] (80.8) |

[a-c] Different letters indicate significant difference at 5% level of probability.

**Table 3.** Mean and increment or decrement percentage (in parenthesis) of physiological attributes in maize genotypes exposed to different water deficit levels.

| Water deficit level | MDA (nmol $g^{-1}$ fresh wt) | Chlorophyll | *RWC* (%) | $H_2O_2$ (µmol/g fr. wt.) | Total soluble proteins (µg $plant^{-1}$ fresh wt) | Yield (g $plant^{-1}$) |
|---|---|---|---|---|---|---|
| Well-watered | 20.246[b] | 43.70[a] | 0.92[a] | 5.95[c] | 1.58[ab] | 142.89[a] |
| Intermediate stress | 24.97[b] (23.3) | 41.14[a] (-5.9) | 0.90[a] (-2.2) | 6.85[b] (15.1) | 1.53[b] (-4.4) | 119.52[b] (-16.3) |
| Severe stress | 34.32[a] (69.5) | 32.94[b] (-24.6) | 0.82[b] (-10.9) | 8.16[a] (37.1) | 1.62[a] (2.5) | 79.10[c] (-44.6) |

[a-c] Different letters indicate significant difference at 5% level of probability.

well-watered irrigation. However, no significant difference was observed between intermediate water-deficit stress and well-watered treatment considering *RWC*, indicating preservation of more water in the plant tissues under stress conditions causing less decrease with increase of stress intensity (severe stress versus well-watered irrigation).

The highest and lowest enzyme activity was detected for isozymes of POX1 and CAT in TWC647 hybrid and Sa0689 line, respectively, under intermediate water-deficit stress (Table 4). The study of enzyme activity changes under intermediate water-deficit stress compared with well-watered treatment (Table 4) showed that the isozyme of POX1 had the highest and lowest activity in Lia0688 (233%), and Sa0689 (-44.4%) lines, respectively. Assessing the rate of changes in the activity of various maize families revealed a positive heterosis for isozymes of CAT and SOD1 belonging to families of 4 (SC647) and 1 (SC706), It means that enzyme activity in F1s (Hybrids) was more than parent lines. Estimated total enzyme activity under intermediate stress for various isozymes showed that POX1 and CAT enzymes had the highest and the lowest activity, respectively, while the isozyme of SOD2 showed totally the highest rate of change activity compared with well-watered treatment, although in some genotypes, POX2 activity showed either a decrement or increment (Table 4).

In case of agro-physiological attributes (Table 5), the highest content of MDA was obtained among the genotypes belonging to MO17 line under intermediate water-deficit stress treatment. In fact, there was no significant difference between genotypes other than the genotype 10, such that most genotypes had the same production of MDA, while the highest content of $H_2O_2$ was detected in 4 and 5 hybrids. Among the genotypes, significant differences were not observed for *RWC*, protein, and chlorophyll content under intermediate stress, besides genotype 10 for *RWC*. Compared to well-watered treatment, assessing the rate of change in content of MDA under intermediate water-deficit stress showed that MDA in AR68 hybrid had the highest increase (93%), while in terms of physiological attributes and grain yield, the B73

line had the highest decrease (-29.2%). Assessing the rate of changes in physiological attributes of various maize families revealed a positive heterosis for the attributes of MDA, leaf chlorophyll, and *RWC* in families of 1 (SC706) and 4 (SC647) for the trait of grain yield in all four maize families assessed. It means that for these traits, the rate of agro-physiological attributes in the F1s generations (hybrids) were more than the parent lines. The estimated total change percentages of physiological attributes under intermediate water-deficit stress condition indicated that the content of MDA and grain yield had the highest and lowest percentage of activity change, respectively, compared with the well-watered treatment (Table 5).

Under severe water-deficit stress (Table 6), the highest enzyme activity was obtained in genotypes TWC647 hybrid and B73 line for isozyme of POX1, and the lowest activity was recorded in line of 10 for isozyme of SOD2. Compared with well-watered treatment, assessing the rate of change in enzyme activity under severe water-deficit stress showed that POX2 isozyme in AR68 hybrid and K1264/1 line had the highest increase (201%) and decrease (-40%), respectively. Studying the rate of change in the enzyme activity in various maize families revealed a positive heterosis in family of 3 (TWC647) for enzymes of POX1, CAT, SOD1, SOD2 and SOD3. The estimated total enzyme activities under severe stress for various isozymes showed that POX1 totally had the highest enzymatic activities in all genotypes, while the isozyme of SOD2 had the higher rate of activity change than well-watered irrigation condition (Table 6).

In comparison of agro-physiological attributes and the rate of changes under severe water-deficit stress condition with well-watered treatment in maize families (Table 7), the highest content of MDA was observed in SC647 hybrid. The rest of genotypes had significantly lower MDA, while the content of $H_2O_2$ was not significant among the genotypes. For *RWC*, three groups were significantly different among the genotypes. Moreover, the assessment of agro-physiological attributes showed that MDA in SC647 hybrid had the highest increase (132.5%), and in TWC647 hybrid, the grain yield had the maximum decrease (-58.1%) compared with the well-watered treatment. Analyzing the rate of change in agro-physiological attributes in various maize families showed that there was a positive heterosis in family of 4 (SC647) for the traits of MDA, leaf chlorophyll, *RWC*, and grain yield. Overall, under severe stress compared with well-watered irrigation, the content of MDA and grain yield had the highest and the lowest percentage of activity change, respectively (Table 7).

Mean comparison of interaction effects of genotype×water deficit condition indicated that in all genotypes, except genotype 8, the activity rate of SOD2 in hybrids was more than parent lines; on the other hands, positive heterosis was observed for hybrids 3, 4, and 5. Accordingly, for SOD2 isozyme in family 1, no change in activity was observed under stress condition compared with the well-watered treatment. In other families, the changes followed a positive correlation, such that the highest increase in activity of SOD2 was recorded for line 2 under severe stress condition, while for genotypes 8 and 10, a significant decrease was detected under the same condition compared with intermediate stress. In contrast, genotypes 2 and 3 showed a significant increase in SOD2 activity with increasing stress intensity. With an increase in stress intensity, SOD3 activity also increased, and genotypes 2, 4, 5, 7, and 8 showed a higher level of activity than the other genotypes. Positive heterosis was also observed for SOD3 activity, With regard to SOD3 activity in family 1 (SC706), only MO17 line showed a significant decrease under severe stress compared with intermediate stress. Moreover, in family 2, the activity level of enzyme in Sa0689 line was lower under intermediate stress than well-watered treatment, while with increase in stress intensity, an increase in activity was observed for SOD3. In the case of CAT, enzymatic activity was orderly increased concomitantly with either an increase or decrease in stress intensity in each of four different maize families. Under intermediate water-deficit stress, the highest level of CAT activity was observed in hybrids 4, 5, and Line 1. Moreover, a positive heterosis was observed





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**Table 4.** Antioxidant enzymes activities in maize genotypes exposed to intermediate water deficit stress compared with well-watered condition. Increment or decrement percentage is shown in parenthesis**.**

| Maize family | Maize genotypes names (Code) | | CAT | POX1 | POX2 | SOD1 | SOD2 | SOD3 |
|---|---|---|---|---|---|---|---|---|
| | K3547/4 (9) | ♀ | 0.0051$^{a}$ (66.7) | 0.025$^{abc}$ (56.2) | 0.0059$^{de}$ (-25) | 0.0085$^{ab}$ (14.3) | 0.0062$^{ab}$ (100) | 0.0059$^{cd}$ (93.2) |
| | MO17 (1) | ♂ | 0.0041$^{b}$ (33.3) | 0.037$^{ab}$ (1) | 0.015$^{bc}$ (-6.3) | 0.0072$^{ab}$ (16.7) | 0.0064$^{ab}$ (75) | 0.0086$^{ab}$ (80) |
| 1 | SC706 (5) | F1 | 0.0061$^{a}$ (50) | 0.033$^{abc}$ (73.7) | 0.0081$^{cde}$ (73.8) | 0.0086$^{ab}$ (80) | 0.0062$^{ab}$ (71) | 0.0078$^{abc}$ (52.9) |
| | Lia0688 (10) | ♀ | 0.0046$^{ab}$ (100) | 0.042$^{ab}$ (223) | 0.0060$^{de}$ (50) | 0.010$^{ab}$ (25) | 0.0076$^{a}$ (100) | 0.0067$^{abc}$ (18.3) |
| | Sa0689 (6) | ♂ | 0.0030$^{b}$ (-25) | 0.015$^{c}$ (-44.4) | 0.0050$^{e}$ (66.7) | 0.0065$^{b}$ (-25) | 0.0038$^{b}$ (33.3) | 0.0039$^{d}$ (-42.8) |
| 2 | AR68 (3) | F1 | 0.0061$^{a}$ (50) | 0.023$^{bc}$ (1) | 0.0055$^{de}$ (100) | 0.0089$^{ab}$ (12.5) | 0.0061$^{ab}$ (50) | 0.0038$^{d}$ (-20) |
| | SC647 (8) | ♀ | 0.0062$^{a}$ (50) | 0.028$^{abc}$ (40) | 0.019$^{ab}$ (11.7) | 0.011$^{a}$ (58) | 0.0077$^{a}$ (167) | 0.0085$^{abc}$ (98) |
| | MO17 (1) | ♂ | 0.0041$^{b}$ (33.3) | 0.038$^{ab}$ (2) | 0.015$^{bc}$ (-6.3) | 0.0072$^{ab}$ (16.7) | 0.0064$^{ab}$ (75) | 0.0086$^{ab}$ (79) |
| 3 | TWC647 (4) | F1 | 0.0054$^{a}$ (2) | 0.045$^{ab}$ (23.1) | 0.026$^{a}$ (13) | 0.010$^{ab}$ (42.8) | 0.0063$^{ab}$ (48) | 0.0093$^{a}$ (125) |
| | B73 (2) | ♀ | 0.0061$^{a}$ (16.7) | 0.023$^{bc}$ (-30.3) | 0.013$^{bcd}$ (-7.1) | 0.0094$^{ab}$ (12.5) | 0.0071$^{a}$ (75) | 0.0091$^{ab}$ (79) |
| | K1264/1 (7) | ♂ | 0.0049$^{ab}$ (25) | 0.032$^{abc}$ (83.3) | 0.014$^{bc}$ (-30) | 0.0086$^{ab}$ (12.5) | 0.0052$^{ab}$ (67.7) | 0.0083$^{abc}$ (100) |
| 4 | SC647 (8) | F1 | 0.0062$^{a}$ (50) | 0.028$^{abc}$ (40) | 0.019$^{ab}$ (11.8) | 0.011$^{a}$ (57.1) | 0.0077$^{a}$ (167) | 0.0085$^{abc}$ (98) |
| Total mean and percentage | | | 0.0052 (37.7) | 0.031 (39.1) | 0.013 (21) | 0.0089 (26.9) | 0.0064 (85.8) | 0.0074 (63.4) |

♀: Maternal line, ♂: Paternal line, and F1: Hybrid.

**Table 5.** Physiological attributes in maize genotypes exposed to intermediate water deficit stress compared with well-watered condition. Increment or decrement percentage is shown in parenthesis.

| Maize family | Maize genotypes names (code) | | MDA (nmol/g fr. wt.) | Chlorophyll | RWC (%) | $H_2O_2$ (µmol/g fr. wt.) | Protein (µg/plant fresh wt.) | Yield (gr/plant) |
|---|---|---|---|---|---|---|---|---|
| | K3547/4 (9) | ♀ | 23.85$^{ab}$ (41.8) | 34.03$^{d}$ (4.9) | 0.88$^{ab}$ (0.4) | 5.57$^{d}$ (-4) | 2.17$^{a}$ (-8.2) | 74.81$^{i}$ (-16.3) |
| | MO17 (1) | ♂ | 28.54$^{a}$ (8.3) | 35.33$^{bcd}$ (-14.8) | 0.91$^{ab}$ (-3.7) | 6.47$^{bcd}$ (39.3) | 1.46$^{b}$ (0.5) | 99.62$^{g}$ (-17) |
| 1 | SC706 (5) | F1 | 26.19$^{a}$ (46.1) | 39.10$^{bcd}$ (-12.8) | 0.92$^{ab}$ (-3.3) | 8.08$^{a}$ (17.6) | 1.59$^{b}$ (14.5) | 206.73$^{a}$ (-26.6) |
| | Lia0688 (10) | ♀ | 17.92$^{b}$ (1) | 38.40$^{bcd}$ (-13) | 0.85$^{b}$ (-9.4) | 5.62$^{d}$ (17.6) | 1.59$^{b}$ (-12.6) | 82.90$^{h}$ (-6.3) |
| | Sa0689 (6) | ♂ | 23.43$^{ab}$ (16.4) | 31.93$^{d}$ (-28.8) | 0.90$^{ab}$ (-4.3) | 7.55$^{abc}$ (68.7) | 1.14$^{c}$ (6.8) | 117$^{e}$ (-8.4) |
| 2 | AR68 (3) | F1 | 26.61$^{a}$ (93) | 36.03$^{cd}$ (-27.8) | 0.91$^{ab}$ (-4) | 6.08$^{d}$ (1.4) | 1.37$^{bc}$ (38.3) | 133.72$^{d}$ (-22) |
| | SC647 (8) | ♀ | 25.09$^{ab}$ (39.2) | 50.86$^{a}$ (12.7) | 0.94$^{a}$ (-2.7) | 6.39$^{cd}$ (-5.6) | 1.60$^{b}$ (-12) | 141.31$^{c}$ (-3.3) |
| | MO17 (1) | ♂ | 28.54$^{a}$ (8.4) | 38.33$^{bcd}$ (-14.9) | 0.91$^{ab}$ (-3.7) | 6.47$^{bcd}$ (39.3) | 1.46$^{b}$ (0.5) | 99.65$^{g}$ (-17) |
| 3 | TWC647 (4) | F1 | 26.19$^{a}$ (90) | 48.90$^{ab}$ (-27.8) | 0.89$^{ab}$ (-4) | 8.44$^{a}$ (45.7) | 1.46$^{b}$ (3.6) | 168$^{b}$ (-25.7) |
| | B73 (2) | ♀ | 24.95$^{ab}$ (1.7) | 40.77$^{abcd}$ (-14) | 0.89$^{ab}$ (-3.6) | 7.87$^{ab}$ (14.4) | 1.48$^{b}$ (6.3) | 65.80$^{j}$ (-29.2) |
| | K1264/1 (7) | ♂ | 23.29$^{ab}$ (24.2) | 46.50$^{abc}$ (1) | 0.89$^{ab}$ (-4.6) | 6.89$^{bcd}$ (0.2) | 1.42$^{bc}$ (-1) | 103.63$^{f}$ (-1) |
| 4 | SC647 (8) | F1 | 25.09$^{ab}$ (39.2) | 50.86$^{a}$ (12.7) | 0.94$^{a}$ (-2.7) | 6.39$^{cd}$ (-5.6) | 1.60$^{b}$ (-12) | 141.31$^{c}$ (-3.3) |
| Total mean and percentage | | | 24.97 (34.1) | 40.92 (-10.2) | 90.25 (-3.8) | 6.81 (19.1) | 1.53 (2.1) | 119.54 (-14.7) |

♀: Maternal line, ♂: Paternal line, and F1: Hybrid.

**Table 6.** Antioxidant enzymes activities in maize genotypes exposed to severe water deficit stress compared with well-watered condition. Increment or decrement percentage is shown in parenthesis.

| Maize family | Maize genotypes names (Code) | | CAT | POX1 | POX2 | SOD1 | SOD2 | SOD3 |
|---|---|---|---|---|---|---|---|---|
| | K3547/4 (9) | ♀ | 0.0057$^{a}$ (100) | 0.029$^{a}$ (81.2) | 0.0077$^{b}$ (1) | 0.0064$^{c}$ (14.3) | 0.0048$^{ab}$ (67) | 0.0059$^{c}$ (100) |
| | MO17 (1) | ♂ | 0.0065$^{a}$ (134) | 0.032$^{a}$ (-3) | 0.022$^{a}$ (37.5) | 0.0095$^{abc}$ (66.8) | 0.0063$^{bc}$ (100) | 0.0074$^{bc}$ (40) |
| 1 | SC706 (5) | F1 | 0.0069$^{a}$ (85) | 0.024$^{a}$ (79.5) | 0.0096$^{b}$ (50) | 0.0093$^{abc}$ (60) | 0.0079$^{ab}$ (107.9) | 0.0058$^{c}$ (45.1) |
| | Lia0688 (10) | ♀ | 0.0056$^{a}$ (200) | 0.025$^{a}$ (92.3) | 0.0067$^{b}$ (75) | 0.0082$^{abc}$ (1) | 0.0046$^{ab}$ (25) | 0.0088$^{abc}$ (49) |
| | Sa0689 (6) | ♂ | 0.0059$^{a}$ (50) | 0.019$^{a}$ (-25.9) | 0.0050$^{b}$ (66.7) | 0.012$^{a}$ (50) | 0.0065$^{bc}$ (100) | 0.0076$^{bc}$ (14.3) |
| 2 | AR68 (3) | F1 | 0.0071$^{a}$ (75) | 0.021$^{a}$ (-8.7) | 0.0087$^{b}$ (201) | 0.011$^{ab}$ (37.5) | 0.0072$^{b}$ (75) | 0.0055$^{c}$ (21) |
| | SC647 (8) | ♀ | 0.0063$^{a}$ (50) | 0.033$^{a}$ (66) | 0.014$^{ab}$ (-17.6) | 0.010$^{abc}$ (43) | 0.0057$^{bc}$ (67) | 0.010$^{ab}$ (149) |
| | MO17 (1) | ♂ | 0.0065$^{a}$ (134) | 0.032$^{a}$ (-3.3) | 0.022$^{a}$ (37.5) | 0.0095$^{abc}$ (66.7) | 0.0063$^{bc}$ (100) | 0.0074$^{bc}$ (40) |
| 3 | TWC647 (4) | F1 | 0.0082$^{a}$ (60) | 0.042$^{a}$ (75) | 0.014$^{ab}$ (-39.1) | 0.012$^{a}$ (71.4) | 0.0073$^{b}$ (76.3) | 0.011$^{a}$ (175) |
| | B73 (2) | ♀ | 0.0065$^{a}$ (17) | 0.042$^{a}$ (27.3) | 0.012$^{b}$ (-14.3) | 0.011$^{ab}$ (37.6) | 0.010$^{a}$ (150) | 0.010$^{ab}$ (99) |
| | K1264/1 (7) | ♂ | 0.0085$^{a}$ (100) | 0.024$^{a}$ (33.3) | 0.012$^{b}$ (-40) | 0.011$^{ab}$ (37.6) | 0.0073$^{b}$ (134) | 0.011$^{a}$ (121) |
| 4 | SC647 (8) | F1 | 0.0063$^{a}$ (50) | 0.033$^{a}$ (65) | 0.014$^{ab}$ (-17.6) | 0.010$^{abc}$ (43) | 0.0057$^{bc}$ (66.7) | 0.010$^{ab}$ (149) |
| Total mean and percentage | | | 0.0067 (87.9) | 0.030 (39.9) | 0.012 (28.3) | 0.010 (44.1) | 0.0066 (89.1) | 0.0084 (83.5) |

♀: Maternal line, ♂: Paternal line, and F1: Hybrid.

**Table 7.** Physiological attributes in maize genotypes exposed to severe water deficit stress compared with well-watered condition. Increment or decrement percentage is shown in parenthesis.

| Maize family | Maize genotypes names (Code) | | MDA (nmol/g fr. wt.) | Chlorophyll | RWC (%) | $H_2O_2$ (µmol/g fr. wt.) | Protein (µg/plant fresh wt.) | Yield (gr/plant) |
|---|---|---|---|---|---|---|---|---|
| | K3547/4 (9) | ♀ | 31.16$^{b}$ (85.3) | 28.23$^{b}$ (-12.9) | 0.86$^{a}$ (-2.1) | 7.95$^{a}$ (37) | 2.51$^{a}$ (6.2) | 67.11$^{f}$ (-24.9) |
| | MO17 (1) | ♂ | 32.59$^{b}$ (25.1) | 33.27$^{ab}$ (-26.1) | 0.91$^{a}$ (-22.8) | 8.20$^{a}$ (76.4) | 1.48$^{cde}$ (1.8) | 54.82$^{h}$ (-54.3) |
| 1 | SC706 (5) | F1 | 29.50$^{b}$ (64.6) | 29.13$^{b}$ (-20.5) | 0.89$^{a}$ (-6.5) | 7.92$^{a}$ (15.3) | 1.79$^{b}$ (29.2) | 131.72$^{a}$ (-53.2) |
| | Lia0688 (10) | ♀ | 28.54$^{b}$ (59.2) | 28.63$^{b}$ (-35.2) | 0.83$^{ab}$ (-11.9) | 8.05$^{a}$ (68.3) | 1.63$^{bcd}$ (-10.2) | 61.84$^{g}$ (-30.1) |
| | Sa0689 (6) | ♂ | 32.67$^{b}$ (62.4) | 39.60$^{a}$ (-11.7) | 0.59$^{c}$ (-37.6) | 8.77$^{a}$ (96.1) | 1.42$^{de}$ (32.6) | 67.10$^{f}$ (-47.5) |
| 2 | AR68 (3) | F1 | 27.57$^{b}$ (100) | 31.60$^{b}$ (-36.7) | 0.81$^{ab}$ (-14.7) | 8.65$^{a}$ (44.4) | 1.36$^{e}$ (38.2) | 101.65$^{b}$ (-40.7) |
| | SC647 (8) | ♀ | 52.37$^{a}$ (132.5) | 36.13$^{ab}$ (-19.9) | 0.88$^{a}$ (-8.9) | 7.98$^{a}$ (17.9) | 1.34$^{e}$ (-26.8) | 96.34$^{c}$ (-34.1) |
| | MO17 (1) | ♂ | 32.59$^{b}$ (25.1) | 33.27$^{ab}$ (-26.1) | 0.91$^{a}$ (-22.8) | 8.20$^{a}$ (76.5) | 1.48$^{cde}$ (1.8) | 54.82$^{h}$ (-54.3) |
| 3 | TWC647 (4) | F1 | 33.50$^{b}$ (100) | 35.93$^{ab}$ (-15.6) | 0.84$^{ab}$ (-14.7) | 8.77$^{a}$ (51.5) | 1.69$^{bc}$ (19.4) | 94.86$^{d}$ (-58.1) |
| | B73 (2) | ♀ | 29.91$^{b}$ (21.9) | 34.03$^{ab}$ (-28.2) | 0.72$^{b}$ (-22.8) | 8.07$^{a}$ (17.3) | 1.89$^{b}$ (35.9) | 46.65$^{i}$ (-49.8) |
| | K1264/1 (7) | ♂ | 28.40$^{b}$ (47.1) | 29.33$^{b}$ (-36.3) | 0.79$^{ab}$ (-15) | 7.40$^{a}$ (22.9) | 1.48$^{cde}$ (1) | 76.32$^{e}$ (-27) |
| 4 | SC647 (8) | F1 | 52.37$^{a}$ (132.5) | 36.13$^{ab}$ (-19.9) | 0.88$^{a}$ (-8.9) | 7.98$^{a}$ (17.9) | 1.34$^{e}$ (-26.8) | 96.34$^{c}$ (-34.1) |
| Total mean and percentage | | | 32.26$^{b}$ (71.3) | 32.94 (-23.9) | 0.83 (-15.7) | 8.16 (45.1) | 1.62 (8.5) | 79.13 (-42.3) |

♀: Maternal line, ♂: Paternal line, and F1: Hybrid.

associated with CAT activity in cultivars 3 and 4, such that CAT activity in these cultivars was higher than parent lines. In family 1, the activity level of parent lines and F1 resulting from them increased with an increase in stress intensity (Figure 4).

Interaction effect of genotype and water-deficit stress associated with agro-physiological attributes showed that the content of MDA increased in all four maize families with an increase in stress intensity, such that the highest MDA content was detected in hybrid 8, and the lowest level in hybrids 5, 3, and line 10. Furthermore, a positive heterosis associated with MDA was only found in genotype SC647 (family 4). This genotype had higher content of lipid peroxidation, even more than the parent lines (Figure 5). Moreover, effect of the increase in stress intensity on the four maize families revealed a decrease in leaf chlorophyll, while the highest level of leaf chlorophyll was observed in genotype 4, under intermediate stress. In Sa0689 line, the level of leaf chlorophyll under severe stress was more than the intermediate stress, although it had no significant difference when compared with well-watered treatment. Compared to other genotypes, leaf chlorophyll content was higher under intermediate stress in hybrids 4 and 8, and under severe stress, in line 6 (Figure 5). Also, with an increase in stress intensity, the decrease of *RWC* under severe stress was higher in the genotypes 2, 3, 6, and 7 compared to the other genotypes. Contrary, in

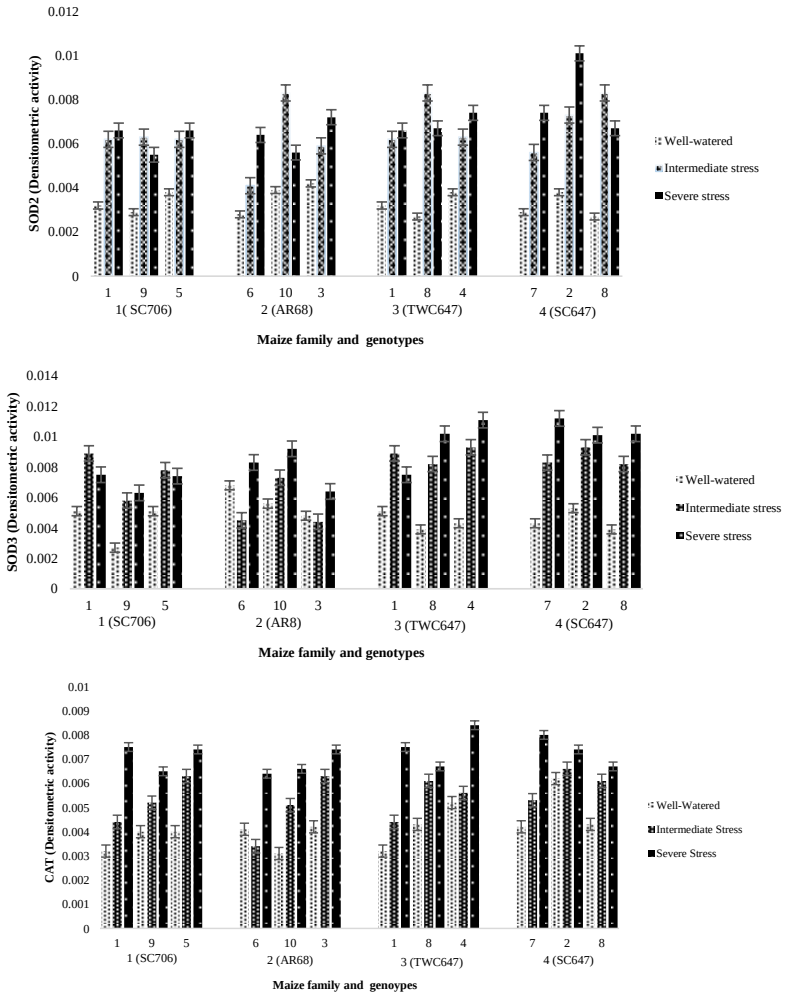


**Figure 4**. SOD2, SOD3 and CAT activity (±S.E) of 12 maize genotypes (Table 1) in four maize families under well-watered, intermediate, and severe water deficit stress conditions.





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the genotypes 8 and 5, the level of *RWC* was higher than the parent lines. In case of *RWC* in four maize families, no significant difference was observed in families 1 and 3 with the increase in stress intensity, while in families of 2 and 4, the decrease in the level of *RWC* was significant concomitantly with an increase in stress intensity. Under severe stress condition, the highest *RWC* was observed in hybrid 5 and line 1, while the lowest was recorded for line 6 (Figure 5).

## DISCUSSION

This study revealed that an increase in water stress intensity in maize from intermediate to severe level was followed by proportional responses including an increase in enzyme activity and the content of MalonDiAldehyde (MDA) and Hydrogen peroxide ($H_2O_2$), and a decrease in grain yield, Relative Water Content (RWC), leaf chlorophyll, and even the soluble protein content. However, various maize genotypes showed different enzymatic and physiological responses under different irrigation treatments. The increase in content of MDA under different stresses showed that water deficit stress through ROSs could lead to lipid peroxidation of different components of the plant (Moussa and Aziz, 2008). The decrease in content of MDA and $H_2O_2$ is in association with tolerance towards drought stress. For instance, the decrease in the content of MDA has been

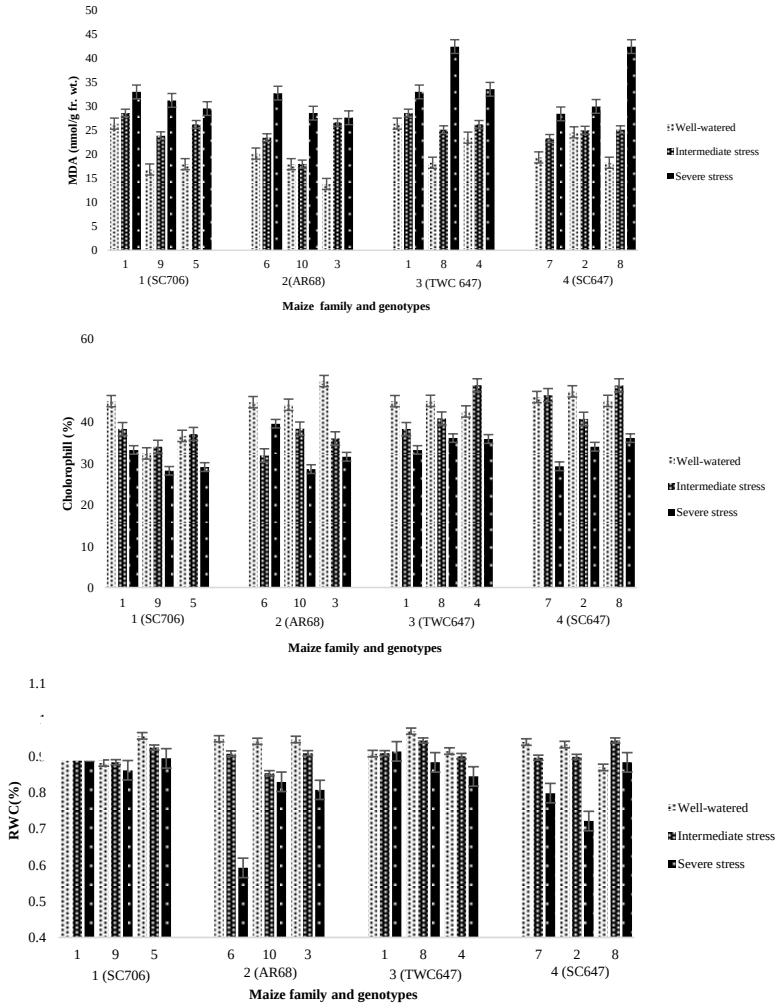


**Figure 5.** MDA, chlorophyll, and *RWC* (±SE) of 12 maize genotypes (Table 1) under well-watered, intermediate, and severe water deficit stress conditions.





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also reported in chickpea (Mohammadi *et al*., 2011) and maize under drought stress (Bai *et al*., 2006; Moussa and Abdel-Aziz, 2008).

Most isozymes assessed in this study showed more activity under water-deficit stress conditions compared with well-watered treatment. The presence of a lot of SODs and POXs in various organs and cytoplasm, and individual CAT located in peroxisome has been reported in most plants (Gaber, 2010). Peroxidase activity in various maize genotypes under water-deficit stresses did not follow a constant process compared with well-watered treatment, however, an increase in activity of POX was observed under water-deficit stress in most genotypes (Figure 2). With an increase in stress intensity, CAT showed higher activity (Figure 1). CAT and POX enzymes are two main antioxidants responsible for scavenging $H_2O_2$ in various intra-cell components (Cruz de Carvalho, 2008). An increase in POX activity in the leaves of resistant and susceptible cultivars of wheat has been reported under drought stress (Sairam and Saxena, 2000). In this study, tolerant genotypes (SC706, TWC647, MO17, B73 and Lia0688) had the highest content of POX activity and membrane stability and the lowest content of lipid peroxidation under stress conditions, while susceptible genotypes (Sa0689 and AR68) showed the lowest level of antioxidant and membrane stability and the highest level of lipid peroxidation. Mean comparison of interaction effect of genotype and water-deficit stress treatment revealed a positive heterosis for CAT in hybrids of TWC647 and SC706 under both intermediate and severe stresses compared with well-watered treatment. With an increase in stress intensity in all four different maize families, CAT activity increased, such that the highest level of CAT activity was observed in TWC647, SC706 and MO17 lines under severe stress. In another study, the seedlings of six maize genotypes [LM5 and Parkash (drought-tolerant), PMH2, JH3459, Paras, and LM14 (drought- susceptible)] were treated under drought stress for 72 h at two-leaf development stages. Enzymes of APX and CAT for all genotypes showed an increase in activity. Superoxide dismutase enzymes showed a significant decrease in susceptible genotypes under drought stress condition, while no change was observed in the tolerant genotypes. In a another study, Moharramnejad *et al.* (2016) indicated that drought stress caused the increase in antioxidant-related enzymes activity and phenolic content in maize seedlings (lines MO17 and B73) under osmotic stress.

In this study, under intermediate and severe water-deficit stress, antioxidant enzymes of CAT, SODs and POXs showed the highest level of activity in hybrids of SC647, SC706, and TWC647. Assessing the rate of activity change for various maize families revealed a positive heterosis for isozymes of CAT and SOD1in family numbers 4 (SC647) and 1 (SC706) under intermediate stress, and for isozymes of POX1, CAT, SOD1, SOD2, and SOD3 under severe stress in family number 3 (TWC647). Superoxide dismutase enzyme is considered as the first line of defense against the adverse effects of the increased level of ROS in plants exposed to stress (Gill and Tuteja, 2010). A high level of SOD enzyme has previously been reported in the resistant inbred lines of maize, compared with susceptible lines (Malan *et al*., 1990). In sunflower and *Aegilops squarrosa* seedlings, the decrease in SOD enzyme has been observed under water-deficit stress (Badiani *et al*., 1990). However, the opposite results (increasing the activity of SOD enzyme) have been reported for wheat (Badiani *et al*., 1990) and rice (Sharma and Dubey, 2005). In the current study, water-deficit stress lead to higher activity of SOD enzyme. The measurement of CAT enzyme in different maize genotypes showed that, with an increase in stress intensity, the level of CAT enzyme activity increased, such that the highest activity was found under severe water-deficit stress. Moreover, mean comparison of interaction effect of genotype and water-deficit stress indicated a positive heterosis for enzymes of SOD2, SOD3 and CAT in hybrids of TWC647 (MO17×SC647) and SC706 (MO17×K3547/4) under both intermediate and severe water-deficit stresses compared with well-watered treatment. Under severe stress, the highest level of activity was detected for B73 line. The results of this study showed that under both intermediate and severe water-deficit stresses, the isozymes of SOD2 and POX2 had the highest and lowest percentage of change, respectively, compared with well-watered treatment. An increase in the level of $H_2O_2$ in





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leaf can be due to increase in SOD enzyme activity (Brou *et al*., 2007).

Considering the agro-physiological attributes assessed under intermediate stress, the line of Lia0688 had lower contents of MDA and $H_2O_2$ than the rest of the genotypes. The MO17 line showed a low level of $H_2O_2$, MDA, and protein content compared with the B73 line. The increase in the activity level of antioxidant enzymes in tolerant cultivars was far more than the susceptible cultivars under various environmental stresses (Wang *et al*., 2009).Contrary, the hybrids of SC647 and SC706 had the highest amount of *RWC*. Under severe water-deficit stress, the lowest level of MDA in hybrid of AR68 and the highest level of *RWC* in line MO17 and hybrid of SC706 were observed. Furthermore, the results of this study indicated that family number 4 (SC647) had a positive heterosis for MDA, leaf chlorophyll, and *RWC*. In case of grain yield, positive heterosis was revealed for all four different maize families as well. The highest level of activity was observed in hybrid SC706 under both intermediate and severe stresses. Moreover, under water-deficit stresses, hybrids SC706 and AR68 and lines MO17 and Li0688 had lower content of MDA, $H_2O_2$ and total soluble protein content. The reduction rate of *RWC* and leaf chlorophyll in these genotypes was lower in stresses, indicating better performance, management, and defense of maize under drought stress. Assessing the interaction effects of genotype and water deficit associated with agro-physiological attributes showed the lowest content of MDA for hybrids SC706 and AR68, and line Lia0688. Also, under intermediate stress, hybrids TWC647 and SC647, and under severe stress, line Sa0689 had the higher levels of chlorophyll. Under severe stress, hybrid SC706 and lines MO17 and Lia0688 showed the highest content of *RWC*. Generally, MDA, *RWC*, and the leaf chlorophyll content are appropriate physiological markers to recognize drought-tolerant cultivars. Given these physiological attributes under severe water-deficit stress, hybrids SC706 and AR68 and line MO17 showed a low level of lipid peroxidation and a high level of *RWC* indicating their higher tolerance to water-deficit stress. The *RWC* index shows the amount of water absorption by textures and cells of plant (Silva *et al*., 2007).

The relative water content has been evaluated as a valuable index for assessment of tolerance to water-deficit stress (Dedio, 1975). Negative heterosis was revealed for $H_2O_2$ in hybrid SC706. The investigations confirm that the levels of these compounds are increased under stress condition, because of the imbalance between production and removal of ROSs. In a study, under water-deficit stress and along with an increase in the level of ABA, an increase in the level of $H_2O_2$ in maize was reported. Previous studies have also demonstrated that $H_2O_2$ acts as a messenger molecule and triggers a cascade of defense reactions in the plants against the stresses (Guan *et al*., 2000). A higher level of leaf chlorophyll content, and *RWC*, and a lower level of MDA and $H_2O_2$ content highlight the higher tolerance of plant spices against environmental stresses, especially drought and water-deficit stresses. Malondialdehyde is produced in result of non-saturated fatty acid peroxidation by reactive oxygen species. It is assumed that the major cause of cellular membrane degradation is production of superoxide radicals ($O^2$-), Hydrogen peroxide ($H_2O_2$) and hydroxyl radicals (OH), which finally leads to the peroxidation of non-saturated fats of the cell membrane (Borsani *et al*., 2001). In this study, only in cultivar TWC647, the level of the total soluble protein was higher than parent lines under severe water-deficit stress. Protein synthesis is a crucial metabolic process leading to improved drought tolerance. Proteins aggregation caused by drought stress leads to physiological compatibility under drought conditions (Hayat and Ahmad, 2007). On the other hand, under water-deficit stress, proteolysis increment and protein synthesis reduction, which cause a decrease in the level of the soluble proteins in the plant, have been reported (Farooq *et al*., 2009).

It can be finally stated that the defense ability of the cells decrease concomitantly with increasing age of leaves, and the level of antioxidant production would be more than their aggregation. Therefore, when plant approaches its final stages of growth, its protection ability is decreased against ROSs. In such conditions, normal activities of cell and then the synthesis of cellular macromolecules such as proteins are disrupted (Swidzinski *et al*., 2004). Furthermore, water-deficit stress leads to an increase in the





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level of $H_2O_2$ and MDA content in susceptible genotypes, while no change has been reported in tolerant genotypes (Chugh *et al*., 2011). The results of this study showed that different maize families and genotypes had different and proportional responses in line with the intensity of water-deficit stress, depending on their genetic structure. In addition, all physiological attributes showed a proportional response with stress intensity and increased activity of antioxidant enzymes was detected for most maize genotypes concomitantly with increasing stress intensity under water-deficit, on the polyacrylamide gel.

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# الگوی نواری آنزیم‌های آنتی‌اکسیدان و ویژگی‌های فیزیولوژیکی در خانواده‌های ذرت (*Zea mays* L.) تحت تنش کمبود آب

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## چکیده

به منظور بررسی اثر تنش کم‌آبی بر فعالیت آنزیم‌های آنتی‌اکسیدان و ویژگی‌های آگروفیزیولوژیکی در برگ‌های ذرت، آزمایشی مزرعه‌ای به صورت اسپلیت‌پلات براساس طرح پایه بلوک‌های کامل تصادفی در سه تکرار پیاده شد. سه سطح آبیاری (نرمال، تنش ملایم و شدید کم‌آبی) به عنوان فاکتور اصلی و چهار خانواده ذرت (شامل ۱۰ ژنوتیپ) به عنوان فاکتور فرعی در نظر گرفته شدند. پس از اعمال تنش‌های کم‌آبی، آنالیز الکترفورتیک سه آنزیم‌های سوپراکسیددیسموتاز (SOD)، کاتالاز (CAT) و پراکسیداز (POX) برگ‌های ذرت با استفاده از ژل‌های ۸٪ آکریلامید افقی انجام گرفت و ویژگی‌هایی آگروفیزیولوژیکی مانند محتوی مالون‌دی‌آلدهید (MDA)، $H_2O_2$، شاخص کلروفیل (SPAD)، محتوی نسبی آب (RWC) و عملکرد دانه مورد اندازه‌گیری قرار گرفتند. با افزایش شدت تنش کم آبی در برگ‌های ذرت اکثر ایزوزیم‌ها و میزان MDA و $H_2O_2$ افزایش یافت ولی فعالیت POX2، میزان کلروفیل، RWC و عمکلرد دانه کاهش یافت. به صورتی که در تنش ملایم ایزوزیم POX1 در لاین Lia0688، ۲۳۳ درصد و در تنش شدید ایزوزیم POX2 در هیبرید AR68 با ۲۰۱ درصد بیشترین افزایش را نسبت به آبیاری نرمال نشان دادند. در حالت کلی ایزوزیم‌های POX1، SOD2وCAT و در میان ویژگی‌های فیزیولوژیکی MDA، کلروفیل برگ، RWC به عنوان ایزوزیم‌ها و ویژگی‌های فیزیولوژیکی برتر شناسایی شدند. براساس فعالیت های آنزیمی و آگروفیزیولوژیکی هیبریدهای SC706 و TWC647 متحمل‌تر و عملکرد بهتری داشتند. همچنین بین لاین‌های والدینی، لاین‌هایMO17 ، B73 و Lia0688 امیدبخش بودند هر چند که در حالت کلی لاین‌های Lia0688 و MO17 نسبت به B73 و سایر لاین‌ها در شرایط نرمال و تنش متحمل-تر و وضعیت بهتری داشتند.





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