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RESEARCH ARTICLE

Variation in oxidative defense system and physiological traits in maize under drought stress

Monireh Shahimoghadam[1], Ali Asghari[1], Sajjad Moharramnejad[2], Zahra Dehghanian[3], Sandeep Kumar Singh[4***], Krishna Moorthy Sivalingam[5] & Senapathy Marisennayya[6*]**

[1]Department of Genetic and Plant Production, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran
[2]Crop and Horticultural Science Research Department, Ardabil Agricultural and Natural Resources Research and Education Center, AREEO, Moghan, Iran
[3]Department of Biotechnology, Faculty of Agriculture, Azarbaijan Shahid Madani University, Tabriz, Iran
[4]Indian Scientific Education and Technology Foundation, Lucknow, Uttar Pradesh, India
[5]Department of Biology, College of Natural and Computational Sciences, Wolaita Sodo University, Post Box No.:138, Wolaita Sodo, Ethiopia
[6]Department of Rural Development and Agricultural Extension, College of Agriculture, Wolaita Sodo University, SNNPR, Wolaita Sodo, Ethiopia

***Email:** drsenapathy@wsu.edu.et; **s.moharramnejad@areeo.ac.ir; ***sandeeps.bhu@gmail.com

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Abstract

The study included the role of key metabolites involved in oxidative defense, and osmotic adjustment under water stress is still undiscovered. We have evaluated whether antioxidant potential could be nominated as a potential marker of drought resistance in three maize hybrids (SC01, SC703, and SC720). Underwater deficiency in all maize hybrids decreased significantly compared to control samples in grain yield, photosynthetic pigments, and phenolic compounds. In contrast, proline and glycine betaine (GB) significantly increased. In contrast, a significant increase ($p < 0.05$) was detected in the lipid peroxidation indicator of malondialdehyde (MDA). The hydrogen peroxide (H_2O_2) and total soluble proteins remained unaffected under drought stress in the three maize hybrids. Electrophoretic investigations attributed three, two, and one isoforms, respectively, to peroxidase (POX), superoxide dismutase (SOD), and catalase (CAT). In the studied maize hybrids, SOD isoforms, including Fe-SOD, Cu/Zn-SOD, and Mn-SOD, appeared on the 8% slab polyacrylamide gels. The water stress decreases Mn-SOD, Cu/Zn-SOD, and Fe-SOD activities in all three hybrids. Further, POX_1, POX_2, and CAT activities decreased in SC01 and SC703, while they increased in SC720 under water deficit stress. In all maize hybrids, oxidative stress from water limitation leads to significant changes in the enzymatic/non-enzymatic antioxidants and main organic osmolytes. Based on the current study's findings, we believe that Cu/Zn-SOD activity, proline, and photosynthetic pigments might be used as biochemical indicators of water stress tolerance.

Keywords

antioxidant; grain yield; hybrid; osmotic adjustment; hotosynthetic pigments

Introduction

Drought stress leads to adverse effects on crop growth and yield. It also influences a broad spectrum of physiological and molecular aspects of plants. Plants have developed several approaches, such as producing compatible solutes to evade the damage caused by water stress (1). Accumulation of organic solutes reduces oxidative damage under drought conditions, safeguards subcellular structures, and maintains enzyme activity (2). Drought stress-induced adverse effects on plant photosynthesis are generally ascribed to a reduction in photosynthetic pigments, reducing the photosynthesis rate (3,4).

Water deficits develop slowly enough to allow changes in developmental processes, as water stress has several adverse effects on plant growth. In this situation, compatible solutes like proline and GB are produced to counter unfavorable cellular conditions. The osmotic potential fluctuation of soil solution creating water stress in plants ultimately leads to plant death due to growth arrest and molecular damage. Osmotic adjustment of cells helps maintain plant water balance and establish an internal milieu (5). Although the definite role of proline and GB in stress resistance is still controversial, they have been designated as advantageous in the osmoregulation and maintenance of enzymes and membranes under stress conditions (9). Moreover, the studies suggest a notable interaction between GB/proline accumulation and plant resistance against stress factors (6,7). The GB and proline contents in maize plants increased under drought stress (8,9).

It is well-documented that reactive oxygen species (ROS) synthesis is increased under drought stress (4). The ROS causes cell membrane dysfunction, protein degradation, and enzyme inactivation by inducing oxidative stress. Drought-tolerant plants can maintain water under drought conditions and show high efficiency against oxidative stress (5). Plants contain numerous antioxidant enzymes, including SOD, POX, and CAT, which reduce toxic compounds or revive their tissues' antioxidants to protect against ROS. During drought conditions, the high activity of antioxidant enzymes may take part in lowering lipid peroxidation, a phenomenon that occurs commonly due to increased ROS production in stress-affected plants (8-10). Non-enzymatic antioxidants with low molecular weight include polyphenols, carotenoids, anthocyanin, ascorbic acid, and glutathione (3). Despite their prominent role as enzyme substrates, they can protect plant cells from oxidative damage (5). For example, dehydration during the pre-and post-flowering stages leads to enhanced activity of different antioxidant enzymes in maize (10). Also, drought stress conditions measured enhanced levels of phenolics and carotenoids in the maize plants (9, 10). Over the past years, several studies have focused on antioxidant defense systems induced by stress conditions. In most cases, a positive correlation has been drawn between drought tolerance and up-regulation of antioxidants (8, 10-12).

Given the above information, we hypothesized that water deficit stress could affect oxidative defense and the pattern of osmotic adjustment in the maize plants. Therefore, the current research was conducted to assess the interconnection of grain yield, antioxidant defense system, photosynthetic pigments, GB, and proline contents in three maize hybrids and whether these antioxidant systems had an association with the degree of drought tolerance in different hybrids of the maize plant.

Materials and Methods

Plant material and experimental conditions

This research included field experiments on three maize hybrids SC01 (TS01×MS02; Sahand Seed Biotechnology Co., Moghan, Iran), SC703 (K47/3×MO17; Seed and Plant Improvement Institute, Karaj, Iran), and SC720 (K47/1×K19; Seed and Plant Improvement Institute, Karaj, Iran). Two individual experiments in 2022 were performed in a randomized complete block design at Moghan Agricultural Research Station, Iran. As the control condition, one investigation was carried out at normal irrigation conditions, and the other was carried out with interrupted irrigation for 14 days ahead of the flowering stage. Four replications were considered for the experiments. Planting of the seeds was carried out on April 20, 2022. The designed plots contained two rows with a length of 3 m and width of 0.75 m, and plant density was 74000 plants/ha. The soil type in the experiments was sandy loam, including 49.8% sand and 18.5% clay. Before planting, the soil features were measured (Table 1). The 25 days following the establishment of the seeds, fertilization was performed by adding urea (60 kg ha^{-1}). In the end, the grain yield per plant was evaluated.

Photosynthetic pigments

The pigment content of the leaves as chlorophyll (a, b), carotenoids, and anthocyanins were determined. The fresh leaf (200 mg) was powdered with liquid nitrogen. The pigment extractions were obtained by adding 2.0ml acetone buffer, including 85% acetone and 15% Tris-HCl (pH=8). The pigment absorbance was read at 470, 537, 647, and 663 nm (9).

Proline and GB

The proline content in fresh leaves of maize was assessed with absorbance read at 520 nm (13). The GB content was performed with absorbance read at 365 nm (14).

Total phenolics

80% acetone was added to the fresh maize leaves, and the mixtures were centrifuged for 10 min at 10,000g. The 100µl volume of the supernatant was mixed with 2 mL water and 1 mL of Folin-Ciocalteau's phenol reagent. Afterward, 5 mL of 20% sodium carbonate was added to the supernatant, and the final volume was increased to 10 mL by adding the

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	195.0	836.0	27.0	3.3	9.9	1.7	1.0

CCE: Calcium Carbonate Equivalent, OM: Organic Matter OM: Organic Mattter; Na:Sodium;K:Potassium;P:Phosphorus; Fe: Iron; Mn:Mangnese; Cu:Copper; Zn: Zinc water. The mixture was thoroughly blended, and absorbance was read at 750nm (9).

Hydrogen peroxide (H_2O_2)

The 0.1% (w/v) TCA (5 ml) was added to 500 mg of fresh leaves, and centrifugation was performed at 12,000g for 15 min. The 0.5 ml of the contents were mixed with 1 ml Potassium iodide (KI, 1 M) and 0.5 ml potassium phosphate buffer (10 mM, pH 7.0). Finally, the absorbance was taken at 390 nm (9).

Malondialdehyde (MDA)

The level of lipid peroxidation was determined in terms of thiobarbituric acid-reactive substances (TBARS) concentration with minor modifications (9). Fresh leaf (1.0 g) was homogenized in 3 mL of 1.0% (w/v) TCA at 4 °C. The homogenate was centrifuged at 20,000g for 15 min, and 0.5 mL of the supernatant obtained was added to 3 mL of 0.5% (v/v) thiobarbituric acid (TBA) in 20% TCA. The mixture was incubated at 95 °C in a shaking water bath for 50 min, and the reaction was stopped by cooling the tubes in an ice water bath. Then, the samples were centrifuged at 10,000g for 10 min, and the absorbance of the supernatant was read at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The concentration of TBARS was calculated using the absorption coefficient, 155 $mmol^{-1}$ cm^{-1}.

Extraction and electrophoresis of antioxidant enzyme

The fresh leaves of maize hybrids were ground with detached mortars and pestles using Tris-HCl (pH 7.5) buffer including PEG 2%, sodium metabisulfite 20 mM, Tris 50 mM, sucrose 5%, ascorbic acid 50 mM, and 2- mercaptoethanol 0.1%. The prepared contents were centrifuged for 10 minutes at 4°C and 10,000g (12). Extract of enzymes was directly attracted into 3×5 mm wicks of Whatman filter paper (3 mm) and was loaded on 8% horizontal slab polyacrylamide gels with TBE electrode buffer (pH 8.8). Total soluble protein was determined in antioxidant enzyme extraction, and electrophoresis was run for 3 hours at 4°C. Electrophoresis was performed for three antioxidant enzymes, including SOD, POX, and CAT, of the maize leaves using 8% slab polyacrylamide gels. To stain SOD isoforms, sensitivity to KCN (2 mM) or H_2O_2 (5 mM) (12) and to stain POX and CAT enzymes was applied (8).

Statistical Analysis

The gels were fixed and scanned immediately after staining. An image analysis program (MCID software) measured each isozyme band's optical density (unit less, D). This program also determined the band area (in2, A). Then D× A (optical density × area) Data were presented as mean ± SE. The Duncan test is applied after performing a one-way ANOVA using SPSS 22.0. Significance in comparing different treatments was determined based on a $p \leq 0.05$.

Results

Water deficiency affected all the maize hybrids by reducing grain yield. The applied water deficit stress decreased the yield of SC01, SC703, and SC720 by about 16.51%, 28.97%, and 47.41%, respectively. The grain yield evaluation indicated that SC01 is considered to be tolerant compared to SC703 and SC720 (Table 2).

Photosynthetic pigment data for control and stressed maize plants are given in Table 3. Water deficit stress diminished the content of leaf pigments, including chlorophyll a, chlorophyll b, anthocyanin, carotenoid, and total chlorophyll concentrations. The reductions were 25%, 36%, 35%, 28%, and 30% for chlorophyll a, b, carotenoid, anthocyanin, and total chlorophyll. Hybrid SC703 had a higher leaf H_2O_2 content than the other hybrids. The photosynthetic pigment evaluation indicated that SC01 had higher chlorophyll a, chlorophyll b, anthocyanin, carotenoid, and total chlorophyll concentrations than SC703 and SC720 under water deficit stress (Table 3).

The content of proline and GB is believed to have a vital role in preventing water loss in plants through cellular osmotic adjustment. The proline and GB concentrations in hybrid maize leaves were enhanced during water deficit stress (Table 2). The augmentation ranged between 29 to 34 percent for proline and 16 to 40 percent for GB accumulation. Generally, the content of proline and GB was the highest in SC01.

Table 2. Mean values and percent inhibition in grain yield, proline, and GB (mean ± SE) in the three maize hybrids.

Hybrids	Grain yield pre-plant (g)			Proline (μmol/g fresh weight)			GB (μmol/g dry weight)		
	Normal	Water deficit	Percent	Normal	Water deficit	Percent	Normal	Water deficit	Percent
SC01	174.79±1.10^a	145.93±2.01^a	-16.51	27.31±2.71^a	36.61±2.43^a	+34.06	12.85±1.13^a	18.03±1.82^a	+40.35
SC703	131.13±1.20^b	93.13±1.79^b	-28.97	26.07±2.82^b	34.01±3.51^b	+31.96	12.43±1.85^b	15.96±1.43^b	+28.39
SC720	118.32±2.11^c	62.22±3.04^c	-47.41	26.14±2.61^b	33.86±1.82^c	+29.64	12.11±1.17^c	14.06±1.22^c	+16.11

Table 3. Pigment contents (mean ± SE) in maize leaves under control and drought stress.

Hybrids		Pigment content (μmol g^{-1} fresh weight)				
		Chlorophyll a	Chlorophyll b	Total Chlorophyll	Anthocyanin	Carotenoid
Control	SC01	0. 22±0.001^a	0. 02±0.0002^d	0. 24±0.008^b	0.130±0.007^a	0.09±0.0006^a
	SC703	0. 22±0.003^a	0. 05±0.0001^a	0. 26±0.004^a	0.123±0.006^b	0.09±0.0006^a
	SC720	0. 19±0.002^b	0. 04±0.0001^b	0. 23±0.003^c	0.115±0.005^c	0.08±0.001^b
Water deficit	SC01	0. 17±0.001^c	0. 01±0.0001^e	0. 19±0.001^d	0.083±0.002^e	0.05±0.0001^d
	SC703	0. 16±0.001^c	0. 03±0.0001^c	0. 17±0.001^e	0.099±0.002^d	0.06±0.0003^c
	SC720	0. 14±0.001^d	0. 02±0.0002^d	0. 15±0.001^f	0.095±0.003^d	0.06±0.0002^c

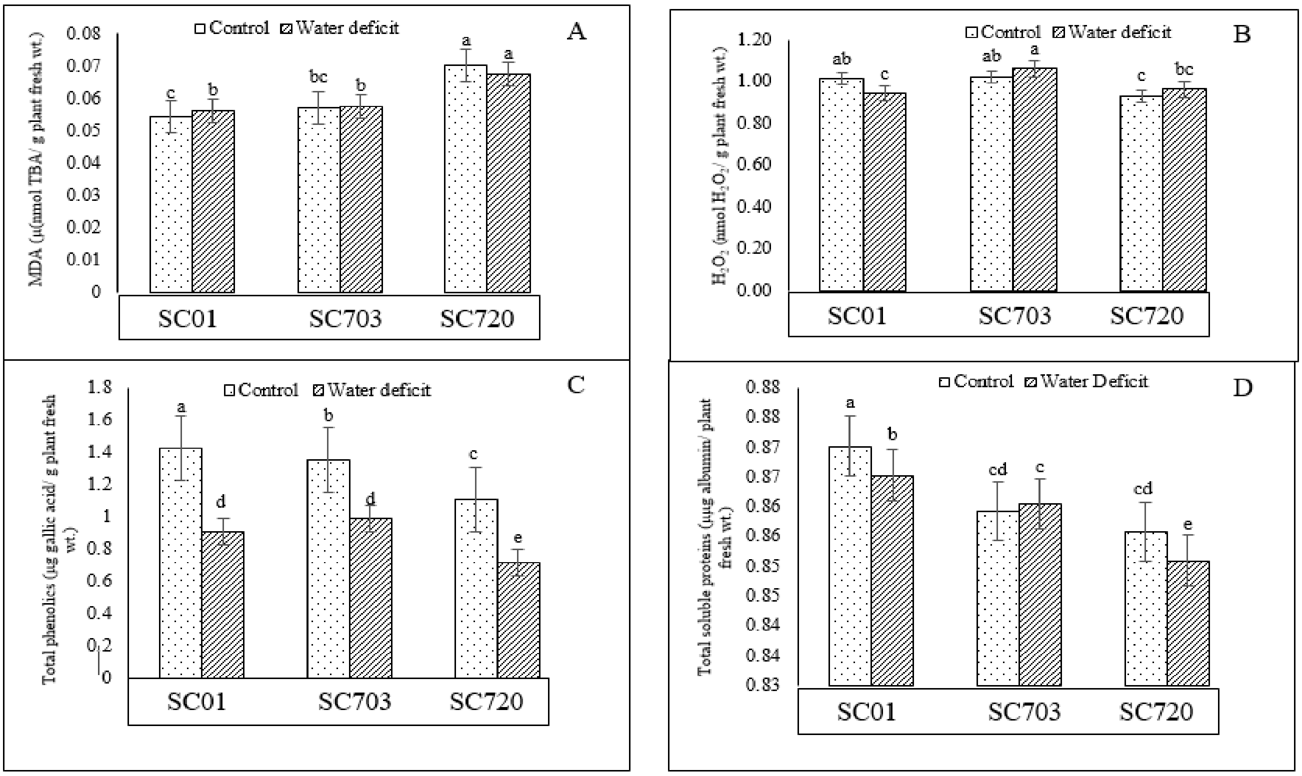

Fig. 1. Means of A-MDA, B-H_2O_2, C-total phenolics, and D-total soluble proteins (±SE) in the three maize hybrids under control and water deficit conditions.

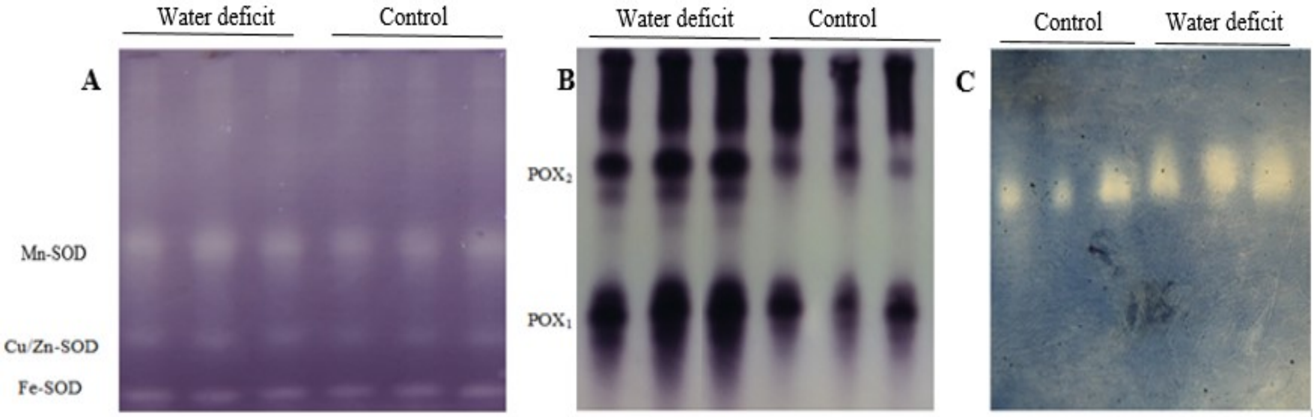

Fig. 2. Example of superoxide dismutase (A), peroxidase (B), and esterase (C) banding pattern for control and water deficit stress conditions in maize.

Under water deficit stress, total phenolic compounds in the leaves of SC01, SC703, and SC720 decreased by about 21.23 percent (Fig. 1). Additionally, complete phenolic content was higher in SC01 and SC703 than in SC720 hybrids under drought conditions.

Although water deficit stress did not significantly affect ($p< 0.15$) leaf H_2O_2 content, it significantly differed among the hybrids (Fig. 1). Hybrid SC703 had a higher leaf H_2O_2 content than the other hybrids. The results show that water deficiency changed MDA content in SC01, SC703, and SC720 hybrids. It was increased in the leaves of SC01 and SC703 and decreased in SC720 hybrid leaves (Fig. 1), though. The highest MDA level was recorded in SC720 under water deficit stress.

The total soluble protein extracted from maize leaves was increased in SC703. In contrast, it was reduced in SC01 and SC720 under water deficit stress (Fig. 1). Total soluble protein was higher in SC01 than in the other two hybrids during the drought.

Based on the results, three, two, and one isoform(s) were determined for respectively SOD, POX, and CAT (Fig. 2). Analysis of variances for the activity of antioxidant isoforms indicated that drought stress and the hybrids significantly affect enzymatic activities in maize. KCN and H_2O_2 were used to find out how the SOD isoforms worked in the polyacrylamide gels. Mn-SOD is resistant to KCN and H_2O_2, whereas Cu/Zn-SOD is sensitive to both inhibitors. Finally, Fe-SOD is resistant to KCN and sensitive to H_2O_2 (Fig. 3).

Under water stress, the activity of all the SOD isoforms as Mn-SOD, Fe-SO, and Cu/Zn-SOD was diminished in maize leaves. Underwater deficiency, Cu/Zn-SOD, Fe-SOD, and Mn-SOD activity were the highest in SC01 and lowest in SC720 maize hybrids (Fig. 3 and 4). The action of POX isoforms (POX_1 and POX_2) was significantly decreased in SC01 and SC703 and was increased in SC720 maize hybrids (Fig. 2 and 4). Water deficit stress significantly modified the CAT activity of all maize hybrids

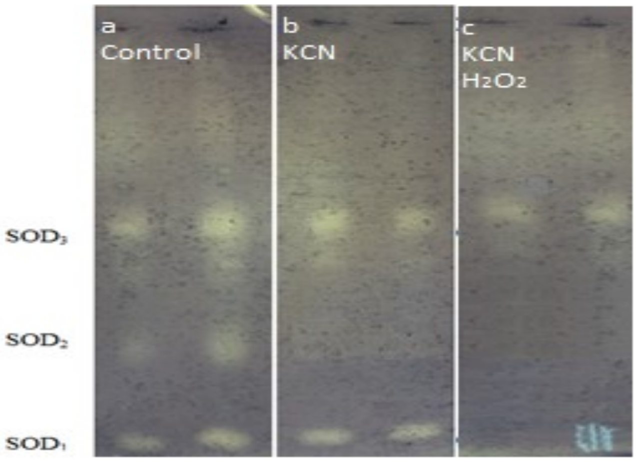

Fig. 3. The activity of SOD isoforms was detected by negative staining and identified based on their sensitivity to KCN and H_2O_2. The Mn-SOD is resistant to both inhibitors; Cu/Zn-SOD is sensitive to both, whereas Fe-SOD is resistant to KCN and H_2O_2.
(a) Control (SOD_1, SOD_2, and SOD_3)
(b) 2mM KCN (SOD_1 and SOD_3): Fe-SOD and Mn-SOD
(c) 2mM KCN and 5mM H_2O_2 (SOD_3): Mn-SOD

(Fig. 2 and 4). CAT activity increased in SC01 and SC703 and decreased in SC720 maize hybrids under stress conditions. The hybrid SC720 exhibited the highest CAT activity.

The data related to the changes in different antioxidant systems are shown in Table 4. As seen, drought-induced fluctuations in the biochemical traits were not constant. They had no relation to the drought tolerance in three maize hybrids formerly characterized as sensitive and tolerant based on grain yield. Therefore, the results indicate maize hybrids' response to an oxidative defense system.

Correlation analysis between grain yield and antioxidant level, osmotic adjustment, photosynthetic pigments, and RWC revealed that grain yield significantly correlates with proline content, Cu/Zn-SOD, and chlorophyll in control and stressed plants. Further, under water deficiency, the grain yield was associated with MDA, H_2O_2, and anthocyanins (Fig. 5). The maize hybrids with high proline accumulation and Cu/Zn-SOD activity detained further reduction in grain yield under water deficit stress.

Discussion

Deteriorated grain yield under drought conditions relates to biochemical, physiological, and molecular procedures controlling the grain yield. In the current study, the three maize hybrids expressed various responses to water deficit stress, possibly due to their genetic diversity, which can affect plant growth (10). Reported 47% inhibition in the grain yield when water deficit stress occurred in the silk emergence (8, 9).

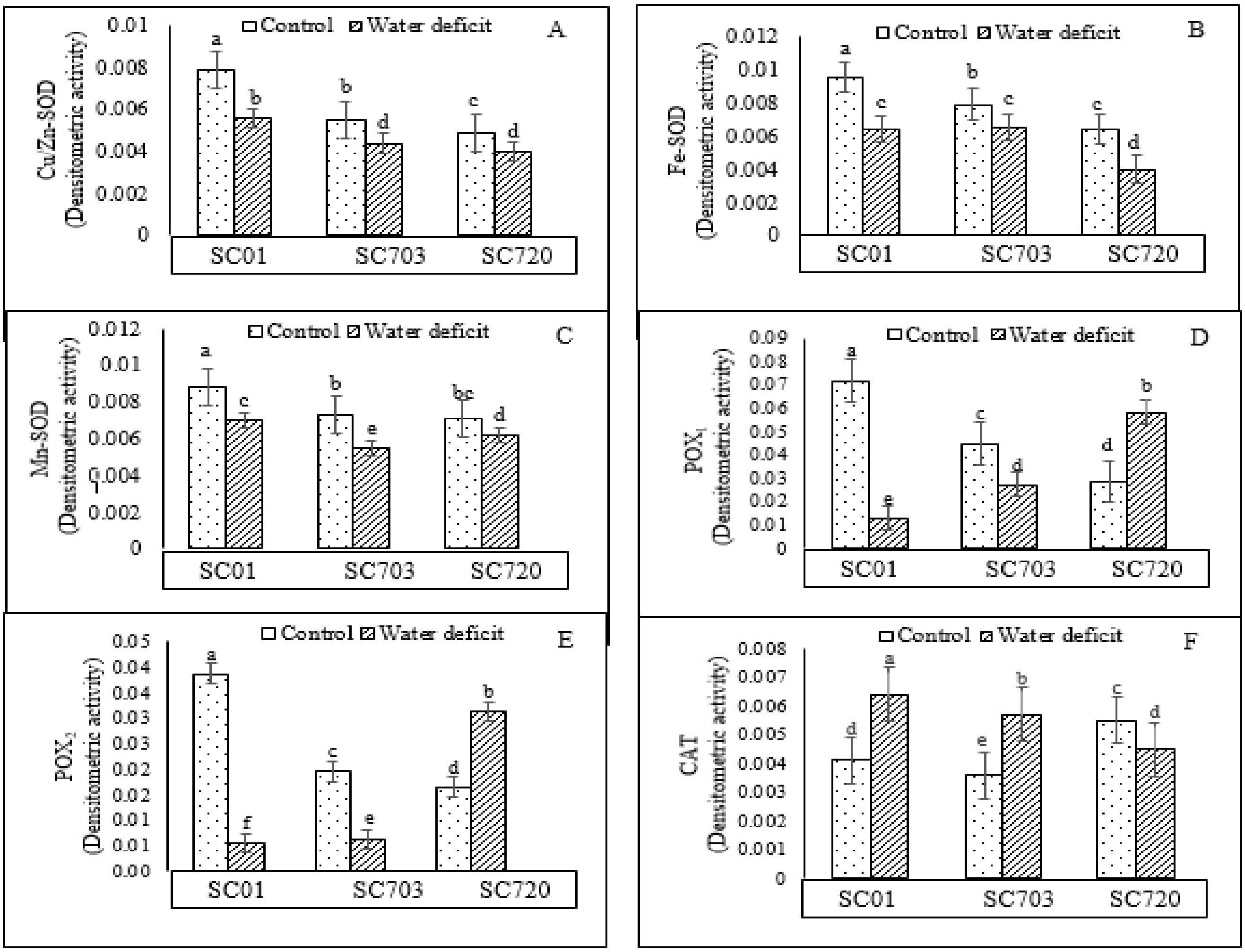

Fig. 4. Means of A-Cu/Zn-SOD, B-Fe-SOD, C-Mn-SOD, D-POX_1, E-POX_2, and F-CAT densitometric activities (±SE) in the three maize hybrids under control and water deficit conditions.

Table 4. Percent inhibition in pigment contents and antioxidant system in the three maize hybrids.

Hybrids	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Anthocyanin	Carotenoid	Total phenolics	H_2O_2	MDA	Total soluble proteins	Fe-SOD	Cu/Zn-SOD	Mn-SOD	POX_1	POX_2	CAT
SC01	24.09	18.19	23.55	35.67	39.08	27.96	7.20	3.55	0.56	29.11	32.63	20.45	82.28	85.75	26.56
SC703	27.44	72.72	35.13	18.62	32.55	18.69	3.37	1.21	0.15	20.00	17.72	24.65	39.37	68.20	36.84
SC720	29.01	56.75	33.91	17.52	22.89	36.31	2.99	3.84	0.51	18.36	37.50	12.67	51.20	47.45	18.18

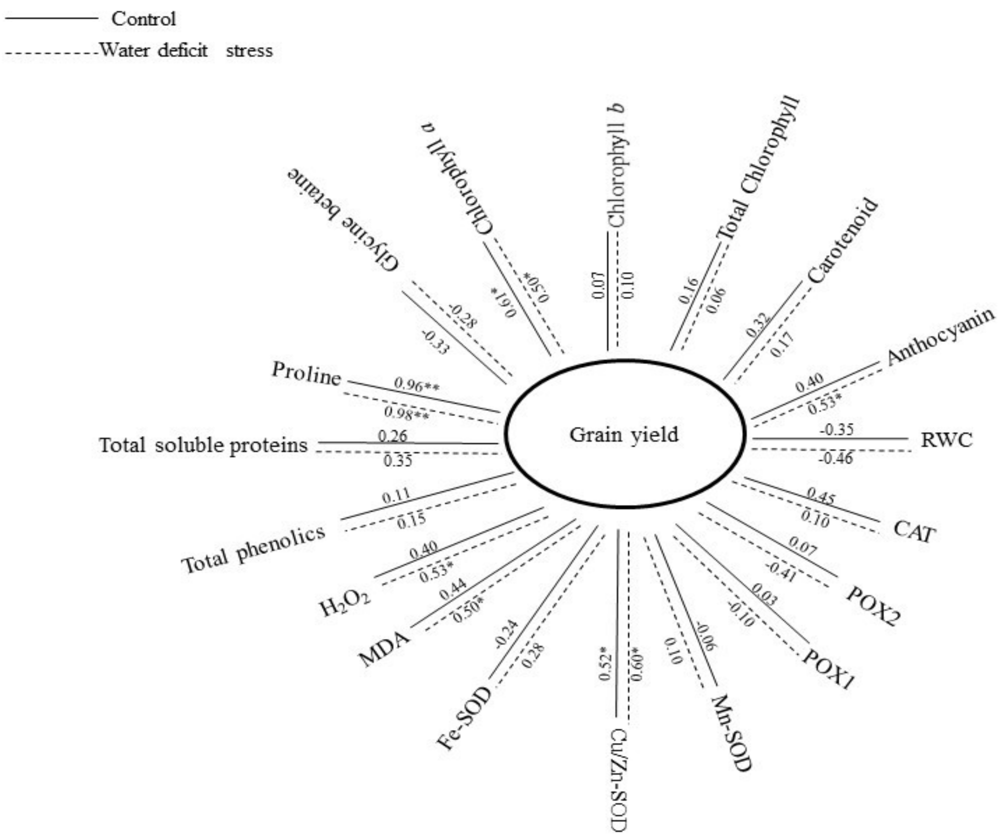

Fig. 5. Correlation between grain yield and antioxidant defense, osmotic adjustment, photosynthetic pigments, and RWC under control and water deficit stress in maize hybrids (* and ** significant at 0.05 and 0.01 levels, respectively).

It is known that drought stress causes substantial damage to photosynthetic pigments and leads to the deterioration of thylakoid membranes (3). Thus, a reduction in photosynthetic capacity in plants exposed to drought stress is expected (9), and the decrease in Chl content is a commonly observed phenomenon (3). Many authors (3, 9, 15) have reported a significant decline in LRWC value in response to drought stress.

In response to water deficiency, proline, which plays a vital role under osmotic stress, is commonly accumulated in the cytosol (16). The current research recorded high proline accumulation for SC01 under water deficit stress. Proline accumulation facilitates the extra intake of water from the surrounding area. This consequently neutralizes water limitation effects (7). Previous reports revealed that applying GB positively affects the growth and grain yield of maize plants stressed by drought conditions (10). Even though the mechanisms by which GB and proline act are not fully elucidated, the application of GB and proline is reported as effective on crop production in stressful environments (9). The content of GB and proline in the maize under water deficit stress showed a significant increase (8). Drought stress improves the accumulation of GB and proline in the leaf, possibly as a response to osmotic adjustment to reduce the drought effect in the plant (1, 7-10).

Out of the secondary metabolites, phenolic compounds are critical to reducing environmental stress effects on the plant (17). Increased phenolic compound synthesis is directly associated with rice abiotic stress (18). In the current research, the total phenolics of the maize hybrids decreased under water deficit stress. In another study, drought stress is reported to reduce the total phenolics of the leaf in maize (8).

Leaf H_2O_2 was unaffected in maize hybrids under water deficit stress. Oxidative stress commonly raises ROS production as $^{\cdot}O_2^{-}$, H_2O_2, and $^{\cdot}OH$ in plants (5). However, the adjustment of ROS is associated with the generation rate (including reaction with metabolites such as nucleic acids, proteins, and lipids) and scavenging and degradation with enzymatic and non-enzymatic antioxidants (4). Commonly, $^{\bullet}O_2^-$ dismutation with antioxidant enzyme produces H_2O_2. Additionally, the peroxisomal photorespiration and -oxidation reactions (3) produce H_2O_2. In the current research, lipid peroxidation as MDA level in the leaves of maize hybrids was different in the three maize hybrids. For example, in SC01 and SC703 genotypes, MDA content increased, while it decreased in SC720 under water deficit stress. An increment of MDA in drought-sensitive (more than drought-tolerant) maize plants affected by drought stress (9).

Three maize hybrids' total soluble protein content did not significantly change under drought conditions. These results are similar to the findings of Yang et al. (19). Enzymatic and non-enzymatic antioxidants preserve plants against ROS by reducing damage. The antioxidant enzyme SOD in aerobic organisms is crucial in diminishing plant ROS effects (20). The classification of SOD isoforms is based on their metal cofactors of iron (Fe-SOD), copper/ zinc (Cu/Zn-SOD), and manganese (Mn-SOD) and which are located in various compartments of the cell (20). Water deficit stress enhanced Mn-SOD activity in the maize hybrids (12). The action of the POX isoforms increased in drought-tolerant and sensitive maize lines under osmotic stress at the seedlings stage (19). CAT activity in SC01 and NS $_{640}$ hybrids was increased under water deficit stress, whereas it was decreased in the SC720 hybrid (Fig. 2 and 3). CAT activity is associated with scavenging H_2O_2 and is promoted with tolerance to abiotic stress (4, 5, 8, 21). It is well-documented that drought stress elevated CAT activity (10). As a result, it can be concluded that the antioxidant defense system and osmotic adjustment, along with the improvement of the photosynthetic system maintained maize productivity under water deficit stress. Enhancing Cu/Zn-SOD activity under drought stress in maize lines (22). When maize plants didn't get enough water, the activity of SOD isoforms and organic osmolytes (GB and proline) was linked to grain yield (8, 9).

Conclusion

In this research, water deficit stress reduced the growth and physiological traits of the three maize hybrids. Overall, the activity of antioxidant enzymes was not consistent in the maize hybrids under water deficit stress. Of the different oxidative defense systems and metabolites, Cu/Zn-SOD activity, chlorophyll a and anthocyanin, and proline correlate with the response of maize hybrids to water deficit stress.

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Authors' contributions

Conceptualization, M.S. and S.M.; methodology, M.S., A.A. and Z.D.; software, M.S., A.A. and Z.D; validation, S.M.; formal analysis, M.S.; investigation, M.S. and S.M.; resources M.S. and S.M.; data curation, M.S. and S.M.; writing—original draft preparation, M.S., S.M. and S.M.; writing—review and editing, S.K.S., K.M.S. and S.M. and; visualization, S.M.; supervision, A.A. and S.M.; project administration, A.A. and S.M. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no competing interests.

Ethical issues: None.

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