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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Alterations of Antioxidative Enzymes and Total Phenolics of Grain Sorghum Seedling Tissues Under Salt Stress

Tuz Stresi Altında Tane Sorgum Fide Dokularında Antioksidan Enzimler ve Toplam Fenolik Bileşiklerdeki Değişimler

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Abstract

Sorghum (*Sorghum spp*) is one of the world's significant warm-climate cereals, used both as animal feed and human food. It demonstrates better adaptation to saline soils and diverse climatic conditions compared to other cereals. Salt stress significantly limits plant growth, development, yield, and quality. This study aimed to investigate the changes in antioxidant defense enzymes and total phenolic compounds in sorghum under salt stress. Different salinity levels (0, 50, 100, 150, and 200 mM NaCl) were applied to the Acme Brom Corn sorghum variety grown in Hoagland nutrient medium. The experiments were conducted under controlled conditions in a climate chamber for 10 days, both under salt stress and non-stress conditions, with three replicates. At the end of the experiments, the roots and leaves of the plants were harvested separately, homogenized, and placed in aluminium foil in portions of 0.5 g and 0.25 g, shock-frozen in liquid nitrogen, and stored at -20 °C. Antioxidant enzymes induced by salt stress, as well as chlorophyll and carotenoids, were determined spectrophotometrically. Statistically significant correlations were found between root glutathione reductase (GR), proline, and ascorbate peroxidase (APX) activities, and between antiradical capacity, glutathione S-transferase (GST), MDA, total phenolics, catalase (CAT), and superoxide dismutase (SOD) activities. In leaves, significant correlations were observed between SOD, total phenolics, and GST, as well as between Chl a, CAT, antiradical capacity, APX, and carotenoids. Significant correlations were also identified between total chlorophyll and carotenoids, GR and Chl b, and MDA and proline. Although increased antioxidant enzyme activity and total phenolics support the salt stress resistance of sorghum plants, these mechanisms were insufficient at high salinity levels. Therefore, it was concluded that further studies are needed to better understand the relationships between salt stress and antioxidant enzyme activities at higher salt concentrations using the investigated sorghum variety. It should be noted that the findings should not be generalized, as the results depend on the experimental year, genotype, salt doses applied, enzyme activities investigated, and methods used.

Keywords: Salinity stress, Catalase, Chlorophyll, Superperoxide dismutase, Glutathione S-tranferase

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Öz

Sorgum (*Sorghum spp*), hayvan yemi ve insan gıdası olarak kullanılan, dünyanın önemli sıcak iklim tahıllarından biridir. Sorgum, tuzlu topraklara ve çeşitli iklim koşullarına diğer tahıllardan daha iyi uyum sağlamaktadır. Tuz stresi, bitki büyümesini, gelişimini, verimi ve kalitesini belirgin şekilde sınırlamaktadır. Bu çalışma, tuz stresi altında sorgumdaki antioksidan savunma enzimleri ile toplam fenol bileşiklerindeki değişiklikleri araştırmak amacıyla yapılmıştır. Hoagland besin ortamında tutulan Acme Brom Corn sorgum çeşidine farklı tuzluluk düzeyleri (0, 50, 100, 150 ve 200 mM NaCl) uygulanarak; kontrollü koşullarda, tuz stresinde ve stres olmadan 10'ar gün iklim kabininde tutulan deneyler 3 tekrarlı yürütülmüştür. Deneylerin sonunda, bitki kökleri ve yaprakları ayrı ayrı hasat edilip, harmanlanmış; 0,5 ve 0,25 g'lık kısımlar halinde alüminyum folyolara yerleştirilmiş, ardından sıvı nitrojende şoklanarak -20 °C'de saklanmıştır. Daha sonra tuz stresi kaynaklı antioksidan enzimler; klorofil ve karotenler spektrofotometrik olarak belirlenmiştir. Kök glutatyon redüktaz (GR), prolin ve ascorbate peroxidase (APX) aktivitesi arasındaki korelasyonlar istatistiki olarak anlamlı bulunmuştur. Antiradikal kapasite, glutatyon S-Transferaz (GST), MDA, toplam fenolikler, katalaz (CAT) ve süperoksit dismutaz (SOD) aktivitesi arasındaki korelasyonlar da anlamlı bulunmuştur. Yaprak SOD, toplam fenolikler ve GST arasındaki, yaprak Chl a, CAT, antiradikal kapasite ve yaprak APX ile Chl a arasındaki, toplam Chl ile karoten arasındaki, GR ile Chl b arasındaki ve MDA ile prolin arasındaki korelasyonlar anlamlı bulunmuştur. Artan antioksidan enzim aktivitesi ve toplam fenolikler, sorgum bitkilerinin tuz stresi direncine destek sağlasa da, bu mekanizmalar yüksek tuzluluk düzeylerinde yeterli olmadığı için tuz stresi ile antioksidan enzim aktiviteleri arasındaki ilişkilerin daha iyi anlaşılması için daha yüksek olan tuz dozlarında, irdelenen karakterler bakımından kullanılan sorgum çeşidinin denemeye alınmasının gerektiği sonucuna varılmıştır. Kuşkusuz elde edilen bulgular genelleştirilememeli, elde edilen sonuçların deneme yılına, genotipe, kullanılan tuz dozlar ile ilgili enzim aktiviteleri, ile uygulanan yöntemlere bağlı olduğu gözden ırak tutulmamalıdır.

Anahtar Kelimeler: Tuzluluk stresi, Katalaz, Klorofil, Süperoksit dismutaz, Glutatyon S-transferaz

1. Introduction

Soil and water salinity are important environmental factors with great impacts on yields and quality in plant production activities (Allakhverdiev et al., 2000; Arzani, 2008). Over 400 million hectares, or roughly 25% of all agricultural lands worldwide, are threatened by salinity (Tilak et al., 2010), and the area of these salty areas is increasing every day (Wang et al., 2003; Munns, 2005; Beyaz and Kazankaya, 2024). Soils have certain dissolved salt quantities. Plants uptake required nutrients among these salts, salt concentrations over normal values and resultant salt accumulation ultimately end up with salinity and restrict plant growth and development significantly (Mahjan and Tuteja, 2005).

Sorghum (*Sorghum spp*) a major cereal crop plant commonly produced for feed and grains (Kardeş et al., 2021). It is more resistant to different environmental and soil salinity than the other cereals genera (Kaplan et al., 2020, Kardeş et al., 2021) Ever-increasing populations and many animals exert a serious pressure on land and water resources. Such a pressure is more remarkable especially in arid and semi-arid regions. As compared to maize (*Zea mays* L), wheat (*Triticum spp*) and paddy (*Oryza sativa*), sorghum has greater drought and salt tolerance (Ayana and Bekele 2000). So, sorghum is considered as a significant alternative to be produced in arid and saline lands to meet continuously increasing animal feed and human food demands (Wambua et al., 2011). Additionally, the production cost of sorghum is lower than that of corn (Esen et al., 2022). Sorghum can easily be grown over saline and arid lands (Boursier and Lauchli, 1990).

Salt stress's effect on plants varies with the plant species, type and quantity of salt applied and exposure durations. Plants exhibit quite different responses to saline conditions just based on their genotypes (Dajic, 2006). Such different responses are observed not only in different species, but also in different cultivars of the same species (Munns, 2002).

Under normal growth conditions, plant antioxidative systems are sufficient for the detoxification of free radicals normally created in plant cells. However, plants activate enzymatic defence mechanisms under stress conditions (Hideg, 1997). Antioxidant enzyme activity generally increases under stress conditions and positive correlations were reported in some cases between plant tolerance to stress factors and enzyme activities (Ozden et al., 2009).

Plants have antioxidants and antioxidative enzymes in different quantities to protect them against the destructive impacts of oxidative damage (Asada and Takahashi, 1987). Protective mechanisms operate to mitigate or minimize such destructive impacts. This protection contains both enzyme based and non-enzyme-based mechanisms. Non-enzymatic antioxidants generally include small molecules like tripeptide glutathione, cystine, hydroxyinons, ascorbate (Vit. C), lyphophilic, vitamin E (α-tocopherol), flavonoids, carotenoid pigments, alkaloids (Larson, 1988). Enzymatic antioxidant defense mechanisms include ascorbate peroxidase (APX) and glutathione reductase (GR) scavenging H_2O_2 respectively in chloroplasts and mitochondria, catalase (CAT) efficiently scavenging H_2O_2 and superoxide dismutase (SOD) scavenging superoxide ions (Scandalios, 1997). The SOD, CAT, APX and GR enzyme activities of salt-tolerant genotypes are quite higher as compared to sensitive genotypes (Yasar et al., 2008).

Proline is an osmotic protector in plants (Djilianov et al., 2005). Proline exists commonly in tall plants and accumulates in greater quantities under salt stress as compared to the other amino acids (Ábrahám et al., 2003). Proline synthesis is an unspecific response to environments with low water potential and thus it is synthesized also under water stress as it was in salt stress (Ashraf and Harris, 2004). Salt stress-induced osmotic stress activates proline synthesis and reduces degradation (Hare et al., 1999). Proline degradation is realized in mitochondria by proline dehydrogenase (PDH) and proline-5-carboxylate dehydrogenase (P5CDH) (Krell et al., 2007). With the removal of stress conditions, proline is oxidized and the resultant ATP and reduced by-products supporting mitochondrial oxidative phosphorylation also reduce stress-induced damages (Hare and Cress, 1997).

This study was conducted to investigate the effects of different salt doses (0, 50, 100, 150 and 200 mM) on carotenes, chlorophyll a, b, and total chlorophyll content, proline, lipid peroxidation and antioxidant enzymes such as CAT, SOD, APX, GR, and glutathione S-transferase activity of sorghum leaves and roots.

2. Materials and Methods

2.1. Plant growth and stress treatments

Acme Brom Corn was used as the plant material of the present study. This material was preferred because of its high yield levels. Experiments were conducted in culture plates (15 cm wide and 6 cm deep) in a climate cabin at 25±1 °C temperature, 70%±5 relative humidity and 16/8 hours of light/dark conditions. Before to placing them into the culture plates, seeds were subjected to surface sterilization by initially washing them with liquid soap and then with ethyl alcohol for 5 minutes and 5% calcium hypochlorite solution for 3 minutes. Seeds were finally washed through distilled water 3 times. Each culture plate had 50 seeds. Plants were grown without any stress factors for 10 days (until they reached a plant height of 10-15 cm), then salt stress was applied for 10 days. Control plants were grown with Hoagland nutrient solution for 25 days and Hoagland solution was frequently supplemented to prevent water stress. Solutions in culture plates (Hoagland in control and salt solutions in treatments) were replenished every day. To create salt stress, 50, 100, 150 and 200 mM NaCl were applied. Following 10-day stress period, plant roots and leaves were harvested, separated into small pieces, blended, placed onto aluminium foils in 0.5 and 0.25 g lots and shocked with liquid nitrogen and frozen at -20 °C. The experiments were conducted in 3 replications, in accordance with the randomized complete block design.

2.2. Crude enzyme extracts and protein concentrations

The method recommended by Misra and Gupta (2006) was slightly modified and used for crude enzyme extracts. About 0.5 g tissue sample was smashed over ice in a pre-cooled mortar with 2 ml of 1% PVP, 0.15 EDTA of 0.1 M potassium phosphate (pH 7.4) buffer. The resultant slurry was centrifuged at 16.000 xg at +4 °C for 20 minutes; supernatant was taken and labelled as crude enzyme extract. Extracts were stored at -20 °C until the analyses. Protein concentrations were determined in accordance with Bradford (1976) method at 595 nm. Bovine serum albumin (BSA) fraction V was used as the standard and the results were expressed in $\mu g ml^{-1}$ protein.

2.2.1. Chlorophyll a, b, total and cretonne contents

Leaf chlorophyll (Chl) and carotene contents were determined in accordance with Hiscox and Israelstam (1979) method. In brief, 100 mg fresh leaf issue was placed into 50 ml tube, supplemented with 7 ml DMSO and kept in a water bath at 65 °C until the removal of color. The liquid portion was taken into a new tube and total volume was completed to 10 ml. Sample optical densities were read against DMSO at 647, 663 and 470 nm. If the OD>0.7, then readings were performed with 50% DMSO dilution. Chlorophyll contents were expressed in mg g^{-1} fresh weight (FW). The following equations were used to calculate chlorophyll contents:

$$Chl\ a = (12.25 \times A\_663) - (2.79 \times A\_647) \quad \text{(Eq. 1)}$$

$$Chl\ b = (21.5 \times A\_647) - (5.1 \times A\_663) \quad \text{(Eq. 2)}$$

$$Chl\ total = (7.15 \times A\_663) + (18.71 \times A\_647) \quad \text{(Eq. 3)}$$

$$Carotene = [(1000 \times A\_470) - (1.82 \times Cl\ a) - (85.02 \times Cl\ b)]/198 \quad \text{(Eq. 4)}$$

2.2.2. Lipid peroxidation (LPO)

Slightly modified version of Madhava Rao and Sresty (2000) method was used to determine the lipid peroxidation of the samples. In brief, 0.5 g fresh tissue was smashed in 5 ml 0.1% TCA solution and the resultant homogenate was centrifuged at 12.000 xg for 5 minutes. Then, 4 ml 20% TCA solution including 0.5% TBA was added for each 1 ml of supernatant. The mixture was placed into screw-cap centrifuge tubes, incubated in boiling water for 30 minutes and cooled with tap water. Tubes were centrifuged at 12.000 x g for 15 minutes and readings were performed in Shimadzu UV-1800 spectrophotometer at OD532 and OD600 nm. In readings, 20% TCA solution including 0.5% TBA was used as blank. Calculations were performed by subtracting OD600 value (background correction) from OD532 value. The molar absorption coefficient (Ɛ) for MDA was 155 $mM^{-1}cm^{-1}$. Results were expressed in TBARS (nmol g^{-1} fw).

2.2.3. Proline

Sample proline contents were determined by Karabal et al. (2003) method. About 0.25 g of fresh tissue was smashed over ice in a pre-cooled mortar with 5 ml 3% sulphuric acid and centrifuged at 5.000 xg and +4 °C for

10 minutes. Of this supernatant, 2 ml was placed into screw-cap tubes and 2 ml acid ninhydrin (1 g ninhydrin was dissolved in 25 ml glacial acetic acid and 16 ml 6 M phosphoric acid) was added and vortexed. Then, 2 ml 96% acetic acid and 1 ml 3% sulphuric acid was added and vortexed. Tubes were placed in boiling water for an hour. They were cooled under tap water, supplemented with 4 ml toluene and roughly vortexed. Tubes were placed in the dark for 1-2 hours for absorption of toluene and readings were performed against toluene at OD520 nm. Besides the samples, 0.01 µM-1.5 mM proline-containing standards (10 standards) were passed through the same processes and the equation of the line obtained from these standards was used to calculate sample proline contents. Results were expressed in nmol g^{-1} fw.

2.3. Enzyme activities

2.3.1. Catalase (CAT)

For catalase activity, 20 mM sodium hydrogen phosphate ($NaHPO_4$) (pH 7.5) buffer, 20 mM H_2O_2 and 50 µl crude enzyme extracts were used. Measurements were made in cooled Shimadzu UV-1800 model spectrophotometer with 2 ml quartz tubs. Buffer was used as blind. The reaction was initiated with the addition of H_2O_2 to the tubs and the decrease in absorbance was monitored at 25 °C for 3 minutes. Molar absorption coefficient of H_2O_2 (Ɛ) was 40 $mM^{-1}cm^{-1}$. Sample specific activity was calculated with the following equation. Results were expressed in unite mg^{-1} protein (Misra and Gupta, 2006).

$$SA = [[\Delta Abs/minute)/40] \times (tub\ volume/crude\ enzyme\ volume) \times (1/protein\ content) * 1000] \quad \text{(Eq. 5)}$$

2.3.2. Superperoxide dismutase (SOD)

Giannopolitis and Ries (1977) method was used for SOD activity. Initially, reaction mixture including 20 mM sodium phosphate buffer (pH 7.5), 0.1 mM EDTA, 10 mM methionine, 0.1 mM NBT and 0.005 mM riboflavin was prepared in a lightproof bottle. Then, a SOD solution including 0.1 mg ml^{-1} SOD was prepared and from these solutions, standard tubes including 10-500 ng ml^{-1} SOD were prepared. Then, 3 ml reaction mixture and 50 µl crude enzyme extracts were placed into screw-cap glass tubes and vortexed. As it was in samples, 3 ml reaction mixture was also added to standards and vortexed. Together with the samples and standards, two extra tubes were prepared only with reaction mixture (light control and blind tube). One of these tubes was wrapped with aluminium foil as to prevent light proofness and the other one was exposed to light same as the other samples. Samples and standards were placed under fluorescent lamps at 20 cm distance for 15 minutes. Then the samples and standards were read against blank in a spectrophotometer at 560 nm. Double tests were performed in 3 replicates. Sample % inhibition values were used in SOD activity calculations. A SOD unit corresponds to an enzyme quantity providing 50% inhibition. The % inhibition values were calculated with the following equation:

$$\%\dot{I}nh = [(light\ control\ absorbance - sample\ absorbance)/\ light\ control\ absorbance] * 100 \quad \text{(Eq. 6)}$$

A logarithmic graph was drawn with % inhibition values vs enzyme concentrations. Then, logarithms of SOD enzyme concentrations were taken, and the same % inhibition values were used to create a new graph. The equation of the line in this new graph was used to determine sample SOD concentrations. Results were expressed in unite mg^{-1} protein.

2.3.3. Ascorbate peroxidase (APX)

For APX activity, 50 mM potassium hydrogen phosphate ($KHPO_4$) (pH 7.0) buffer, 0.15 mM ascorbic acid and 20 mM hydrogen peroxide (H_2O_2) were used as reaction medium. Then, 50 µl crude enzyme extract was added to this reaction medium and decrease in absorbance was monitored in 2 ml tubs of spectrophotometer at 290 nm for 3 minutes. Buffer was used a blind and reaction was performed in quartz tubs at 25 °C. The molar absorption coefficient of H_2O_2 at 290 nm (Ɛ) was 2.8 mM^{-1} cm^{-1}. The sample-specific activity was calculated with the following equation. Results were expressed in unite mg^{-1} protein (Akbulut and Çakır, 2010).

$$SA = [[(\Delta Abs/minute)/2.8] \times (tub\ volume/crude\ enzyme\ volume) \times (1/protein\ content)] * 1000 \quad \text{(Eq. 7)}$$

2.3.4. Glutathione reductase (GR)

GR activity was determined by Misra and Gupta (2006) method. The reaction was initiated by adding 50 µl crude enzyme extract to 100 mM potassium phosphate ($KHPO_4$, pH 7.5) buffer including 0,1 mM Na_2EDTA, 0.1 mM nicotine amide adenine dinucleotide phosphate (NADPH) and 1 ml oxide glutathione (GSSG) and the decrease in absorbance was monitored in 2 ml tubs at 25 °C for 5 minutes. Buffer was used as blind and the reaction was performed in quartz tubs. Molar absorption coefficient of NADPH at 340 nm (Ɛ) was 6.2 mM^{-1} cm^{-1}. The sample-specific activity was calculated with the following equation. Results were expressed in unite mg^{-1} protein.

$$SA = [[(\Delta Abs/minute)/6.2] \times (tub\ volume/crude\ enzyme\ volume) \times (1/protein\ content)] * 1000 \quad \text{(Eq. 8)}$$

2.3.4. Glutathione S-tranferase (GST)

GST activity was realized in 100 mM potassium phosphate ($KHPO_4$, pH 7.5) buffer containing 0.1 mM EDTA, 0,1 mM NADPH, 1 mM (GSH) and 1 mM (CDNB). Reaction medium was supplemented with 50 µl crude enzyme extract, end of non-specific activity was waited for 5 minutes and the decrease in absorbance was monitored in 2 ml quartz tubs at 25 °C and 340 nm for 5 minutes. Molar absorbance coefficient of NADPH at 340 nm (Ɛ) was 6.2 mM^{-1} cm^{-1}. Sample specific activity was calculated with the following equation. Results were expressed in unite mg^{-1} protein (Yilmaz et al., 2017)

$$SA = [[(\Delta Abs/minute)/6.2] \times (tub\ volume/crude\ enzyme\ volume) \times (1/protein\ content)] * 1000 \quad \text{(Eq. 9)}$$

2.4. Statistical Analyses

Variance and correlation analyses were performed by using SAS software (SAS Inst., 1999). Differences between mean values were tested by Duncan's multiple range test. In order to visualize the relationships among traits in the same chart, bi-plot analysis was performed by using Microsoft excel software as suggested by Lipkovich and Smith (2002).

3. Results

3.1.Effects of salt treatments on chlorophyll and carotene content

The effects of salt treatments on carotene, contents were found to be highly significant ($p \leq 0.01$) and Chl a, Chl b and Total Chl was significant ($p \leq 0.05$). Carotene contents increased with increasing salt levels, but the greatest value was obtained from 50 mM treatment and 100 mM treatment was also placed into the greatest group. The highest chlorophyll a content was observed at the 100 mM dose, which was statistically grouped with the 0 mM and 50 mM doses. Similarly, the highest chlorophyll b content was recorded at the 100 mM dose, sharing the same statistical group with the 50 mM, 150 mM, and 200 mM doses. The highest total chlorophyll content was also obtained at the 100 mM dose. Beyond 100 mM, a decline in chlorophyll content was observed. (*Table 1*).

Table 1. Chlorophyll and carotene content upon exposure to salt stress

Salt Treatment	Chl a*	Chl b*	Total Chl*	Carotene**
0 mM	908.37a	168.48b	1076.80ab	136.39b
50 mM	823.54ab	292.23ab	1115.80ab	202.80a
100 mM	913.00a	429.37a	1342.40a	193.16a
150 mM	672.00bc	323.14a	995.10b	149.26b
200 mM	565.11c	313.70ab	878.80b	134.68b
LSD	**215.32**	**148.37**	**308.59**	**38.379**

* $p \leq 0.05$; ** $p \leq 0.01$; **LSD:** Least Significant Difference

3.1.1. Effects of salt treatments on proline accumulation

The effects of salt treatments on root and leaf proline contents were found to be highly significant ($p \leq 0.01$). Root proline contents increased with increasing salt levels until 150 mM, but decreased again in 200 mM treatment. Leaf proline contents regularly increased with increasing salt doses (*Figure 1*).

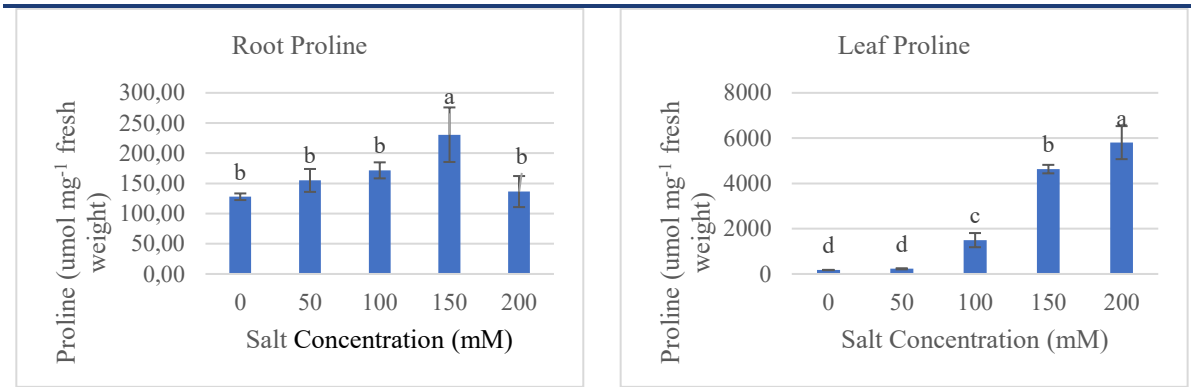

Figure 1. Proline levels in leaf and root tissues of sorghum under salt stress

3.1.2. Effect of salt treatments on LPO

Salt treatments significantly affected on both root and leaf LPO activity ($p \leq 0.01$). Leaf LPO activity regularly increased with increasing salt doses, but root LPO activity increased until 100 mM and decreased later on (*Figure 2*).

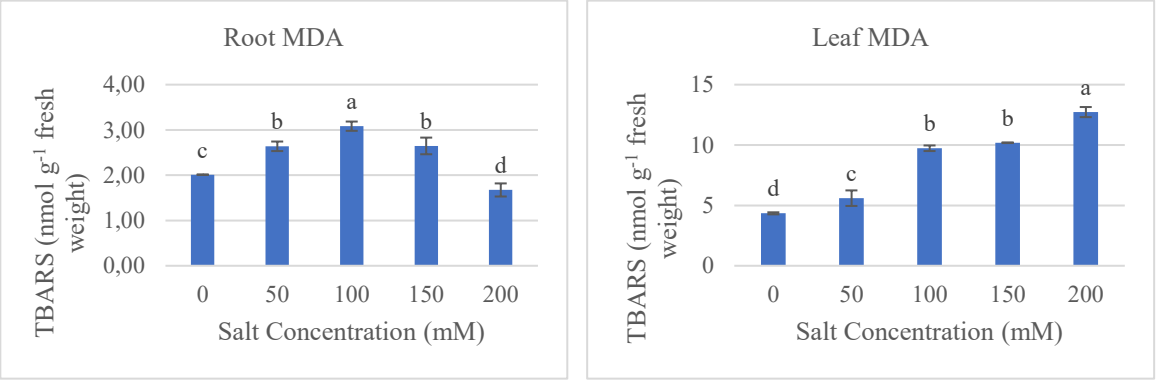

Figure 2. MDA levels in leaf and root tissues of sorghum under salt stress

3.2. Effect of salt treatments on antioxidant enzymes

3.2.1. SOD

A steady decrease was observed in root and leaf SOD activity with increasing salt doses above 100 mM. The differences in root and leaf SOD activities of salt doses were found to be significant ($p \leq 0.05$) (*Figure 3- 4*).

3.2.2. CAT

The effects of salt treatments on root and leaf CAT activity were found to be highly significant ($p \leq 0.01$). The greatest root and leaf SOD activity was obtained from 100 mM salt treatment (*Figure 3-4*).

3.2.3. APX

Salt treatments had highly significant effects on both root and leaf APX activity ($p \leq 0.01$). As it was in CAT activity, again the greatest root and leaf APX activity was obtained from 100 mM treatment. However, treatment-induced changes in leaf APX activities were not found to be significant and they were all placed into the same statistical group (*Figure3-4*).

3.2.4. GR

The effects of salt treatments on GR activity of sorghum plants were found to be significant ($p \leq 0.05$). The greatest root GR activity was obtained from the 150 mM treatment. The other treatments were statistically placed into the same group. The effects of salt treatments on leaf GR activity were found to be highly significant ($p \leq 0.01$). Although the greatest leaf GR activity was obtained from the 100 mM treatment, the activities of the 150 and 200 mM treatments were also placed into the same statistical group (*Figure 3-4*).

3.2.5. GST

Concerning GST activity, roots and leaves had different responses to salinity. Salinity treatments had highly significant effects on the GST activity of both the roots and the leaves ($p \leq 0.01$), but leaf GST activity linearly decreased with increasing salt doses, root GST activity initially increased and decreased later (*Figure 3-4*).

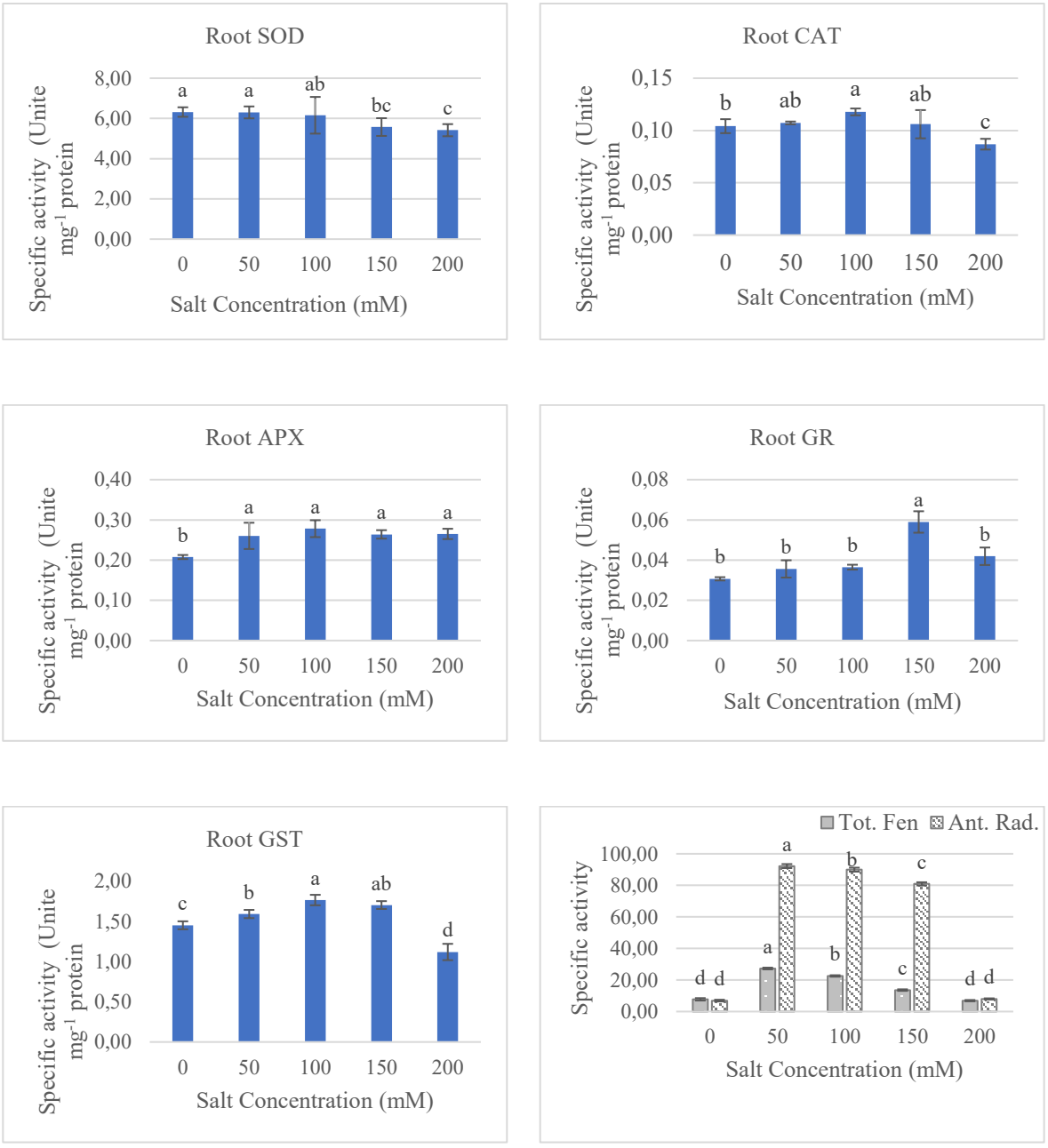

Figure 3. Antioxidative enzyme activities, total phenolic and antiradical capacity in root tissues of sorghum under salt stress

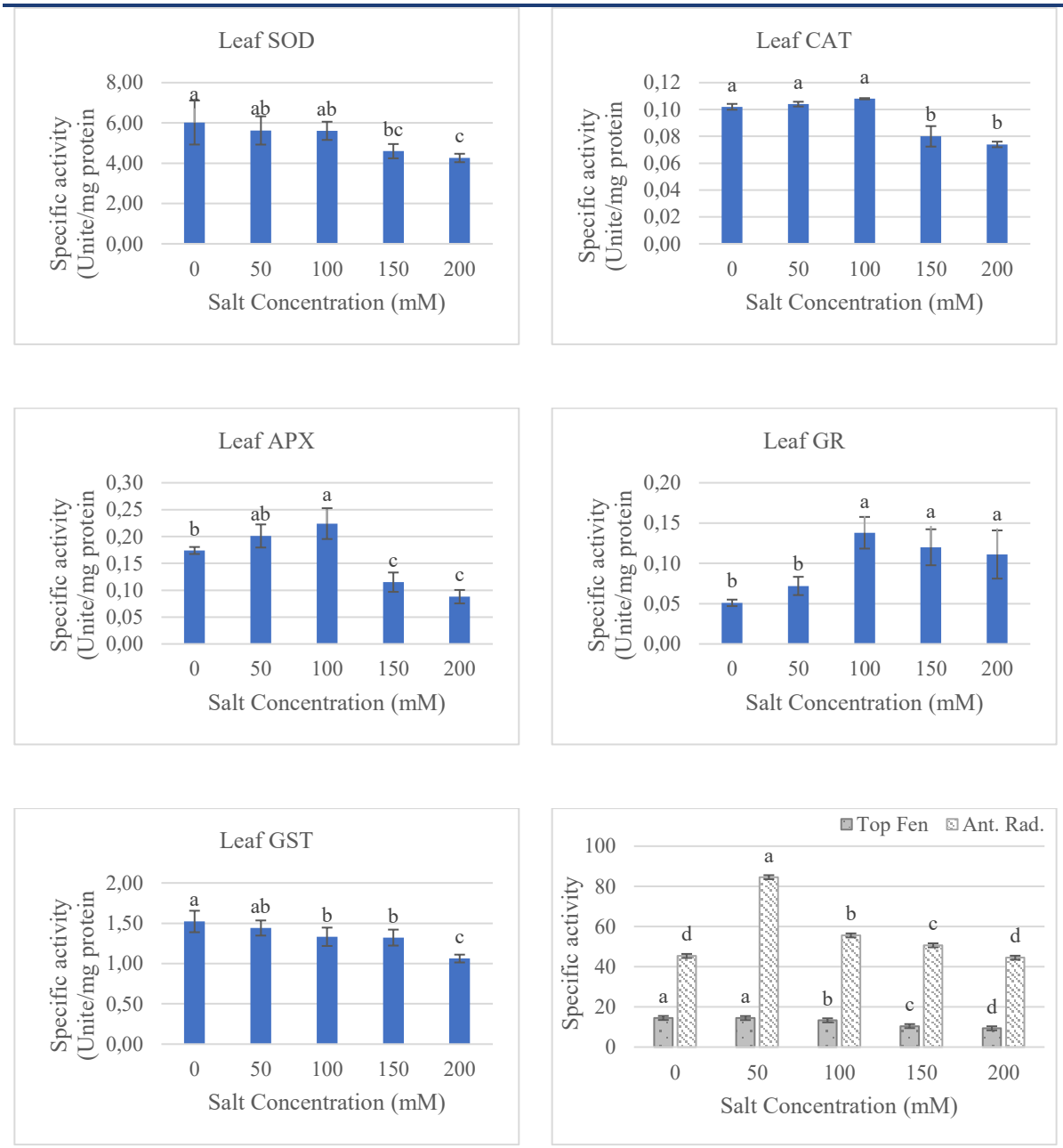

Figure 4. Antioxidative enzyme activities, total phenolic and antiradical capacity in leaf tissues of sorghum under salt stress

3.3. Relationship among antioxidant enzymes, Proline, MDA, total phenolics and antiradical capacity

Biplot graph explained 77% of the variation in root and 80% of the variation in leaf (*Figure 5* and *6*). Such explanation values were quite for a genotype – trait biplot graph. Therefore, it was through that group-based assessment would be more reliable in this case. The biplot graph drawn for root genotype – trait relations revealed two groups (*Figure 5*). The first group was composed of GR, proline and APX and the correlation coefficients among these 3 traits were found to be significant. The second group was composed of antiradical capacity, GST, MDA, total phenolics, CAT and SOD and the correlations coefficients among these 6 traits were found to be significant. The biplot graph drawn for leaf genotype - trait relations revealed 5 groups (*Figure 6*). The first group was composed of SOD, total phenolics and GST. The correlation coefficients among these 3 traits were found to be significant. The second group was composed of Chl a, CAT, antiradical capacity and APX and the correlation coefficients among these traits were found to be significant. The third group was composed of Total Chl and carotene and the correlations coefficients between these two traits were found to be significant.

The fourth group was composed of GR and Chl b and the correlation coefficients between these traits were found to be significant. The fifth group was composed of MDA and proline (*Figure 6*).

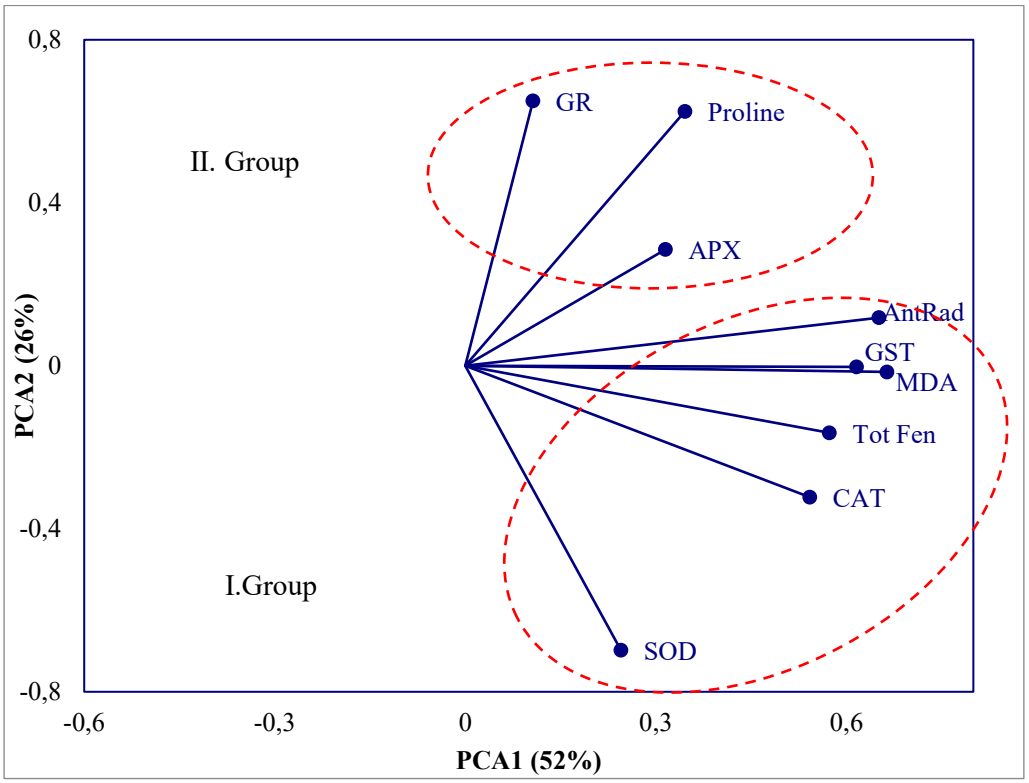

Figure 5. The biplot polygon for antioxidative enzyme activities in sorghum root under salt stress

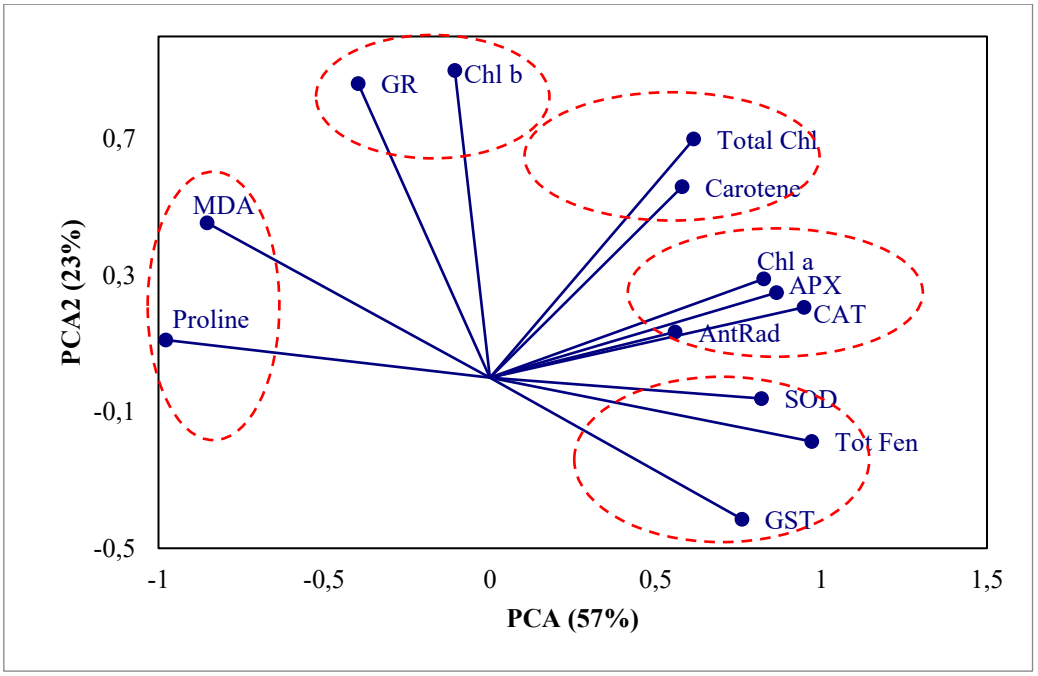

Figure 6. The biplot polygon for antioxidative enzyme activities in sorghum leaves under salt stress

4. Discussion

In sorghum, photosynthesis primarily occurs in the leaves. The organelle most closely associated with photosynthesis is the chloroplast, which is more sensitive to salt stress. Salt stress disrupts the structure of chloroplasts, reducing chlorophyll content in the plant and hindering the process of photosynthesis (Liu et al.,

2023). Yin et al. (2016) reported that the activities of SOD, CAT, POD, and APX in sorghum seedlings under salt stress increased with rising salt concentrations, particularly in salt-tolerant varieties. They also stated that sorghum seedlings exhibited greater resistance to salt stress by enhancing antioxidant enzyme activities, thereby preventing excessive ROS accumulation and lipid peroxidation in the cell membrane.

It has been found that the soluble protein, soluble sugar, and betaine contents of sorghum leaves increase, while organic acids accumulate in the roots under salt stress (Wang et al., 2022). Compared to salt-sensitive crops such as rice and maize, osmoregulatory substances are significantly reduced. This indicates that the accumulation of organic osmotic substances in sorghum collectively contributes to resistance against salt stress-induced plant damage, while salt-sensitive varieties lose part of their protection provided by organic osmotic substances (Liu et al., 2023).

Chl a, b and total chl contents of salt-tolerant plants are expected to increase gradually with increasing salt concentrations, but such values of salt-sensitive plants are expected to increase parallel to increasing salt concentrations (Akram and Ashraf, 2011). Therefore, chlorophyll accumulation was accepted as potential biochemical indicator for salt tolerance of peas (Noreen et al., 2010) and sunflowers (Khan et al., 2009). In present study, chlorophyll and carotenoid content of sorghum plants increased until 150 mM salt treatment and decreased later. Decrease in chlorophyll contents was probably resulted from salt-induced increase in chlorophyll degradation enzyme activity (Noreen and Ashraf, 2009) or decrease in chlorophyll-precursor 5-aminolevulinic acid (ALA) content (Santos, 2004). Although total chlorophyll and carotenoid content of plant leaf tissues generally decrease under salt stress because of negative impacts of salt on membrane stability (Agastian et al., 2000; Ashraf and Bhatti, 2000), some studies reporting increasing chlorophyll ca contents at low salt concentrations (Hossain et al., 2006). It was also pointed out that increase in chlorophyll a/b ratio was a significant parameter for the salt resistance of the plants (Oncel and Keles, 2002).

Root and leaf proline contents increased with increasing salt stress. Accumulation of compatible organic soluble substances, also called osmolites, is a common plant response to salt stress. Carbohydrates, amino acids, organic acids and proline are the most common compatible organic soluble substances (Hasegawa et al., 2000). Proline content of sorghum plants generally increase under salt stress (Lacerda et al., 2001). Molecular studies revealed that salt stress stimulated proline synthesis (Sharma and Verslues, 2010). Although proline accumulation increased under salt stress in salt-tolerant and sensitive sorghum genotypes, proline contents generally varied based on salt tolerance levels of the plants and salt stress levels (Lacerda et al., 2003). Proline stabilizes proteins, membranes and sub-cellular structures, scavenges ROS, and thus protects cellular functions (Reddy et al., 2015).

Salt stress may have negative effects on dark reactions of photosynthesis, damage lipid membranes, and the results in excessive ROS formation, high MDA levels and severe leaf damages (Moradi and Ismail, 2007). High TBARS levels, as a product of lipid peroxidation, indicate free radical damages against cell membranes creating serious oxidative stress (Metwally et al., 2004). It was reported in previous studies that salt stress negatively influenced lipid peroxidation in *Brassica juncea* (Ahmad et al., 2012), *Vicia faba* (Azooz et al., 2013), *Solanum tuberosum* (Queiros et al., 2011) and *Cicer arietinum* (Rasool et al., 2013). MDA is commonly used as a selection criterion to assess salt damage in various plants (Ahmad et al., 2011, 2012). It was reported in previous studies that there were significant negative correlations between proline levels and MDA production, free radical production increased under salt stress (Alia Saradhi and Mohanty, 1993) and high proline accumulation decreased free radical levels (Vendruscolo et al., 2007). Cherian and Reddy (2003) asserted that proline prevented free radical-induced damage on membranes under salt stress. It was reported that low lipid peroxidation levels resulted from high antioxidant activities provided resistance to salt stress in tomatoes (*Solanum lycopersicum*) (Shalata and Tal, 1998), cotton (*Gossypium hirsitum*) (Gossett et al., 1994) and wheat (*Triticum aestivum*) (Dionisio-Sese and Tobita, 1999).

ROS scavenging activity of antioxidant enzymes relieve antioxidative stress and such a mechanism is a significant plant strategy to improve stress tolerance of plants (Reddy et al., 2015). Positive correlations were reported between proline accumulation and antioxidant enzyme activities of tobacco (*Nicotina tabacum*) plants under salt stress (Hoque et al., 2007). In present study, significant changes were observed in antioxidant enzyme activities of sorghum plants under salt stress. Bor et al. (2003) reported that antioxidant enzymes provided better protection against salt stress-induced oxidative damage and POD, APX, CAT and GR activities played significant

roles in this protection and SOD had greater activity than the other enzymes. SOD and CAT are the most efficient antioxidant enzymes in prevention of cellular damage (Scandalios, 1993). SOD is responsible for scavenging of superoxide radicals and it is critical enzyme accepted as a significant antioxidant in aerobic cells. Combined effect of SOD and APX is a critical issue in relieving the impacts of antioxidative stress, since the former merely acts on the superoxide intermediate (H_2O_2) and the latter acts on H2O2 converting anion into water and oxygen (Rasool et al., 2013). Increases were observed in SOD and APX activities in this study. CAT and SOD scavenge H_2O_2 produced with the other reactions (Foyer et al., 1994). Under salt stress, increasing CAT activities were also reported in tomatoes by Latef and Chaoxing (2011), in *Brassica juncea* by Mittal et al. (2012), in paddy seedlings by Nounjan et al. (2012) and in soybeans by Arshi et al. (2012). Since APX and GR are the key enzymes of the ascorbate - glutathione cycle (Noctor and Foyer, 1998), this pathway could be a potential mechanism for sorghum to salt stress. Similar with the present findings, under salt stress, increasing APX activities were reported in paddy seedlings by Nounjan et al. (2012), in *Vigna radiata* by Nazar et al. (2011), in tomatoes by Latef and Chaoxing (2011) and in *Brassica juncea* by Mittal et al. (2012).

5. Conclusions

Preserved chlorophyll levels and increased carotene contents under increasing salt doses may be considered as the initial response of sorghum plants to stress conditions. Then, another response was provided over the antioxidant enzymes. Antioxidant response is formed over a couple of alternative pathways. As can be seen from the bi-plot graphs, such antioxidant responses emerged separately over CAT-APX, SOD-GST and GR. Besides these responses, proline accumulation and antiradical capacity increased to prevent stress-induced damage of reactive species on the membrane. Despite all these responses to stress factors, the reactions of sorghum plants weakened under high stress levels and were not sufficient for total protection against salt damage. Increasing antioxidant enzyme activity and phenolics content under stress conditions should encourage researchers to conduct new studies about the relationships between phenolics and enzyme activity. Considering the relationships among the investigated traits revealed in bi-plot graphs, further studies are recommended to be done with a single attribute of each group. Researches are also recommended to be done on the effects of abiotic stress conditions on the phenolics composition of sorghum plants and to identify efficient phenolics.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Kaplan, M.; Design: Temizgül R, Yılmaz S.; Data Collection or Processing: Kardeş Y. M., Çiftçi B.; Statistical Analyses: Varol, İ. S.; Literature Search: Kaplan M.; Writing, Review and Editing: Kaplan M., Kardeş Y. M.

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