

Article



Morphological, Physiological and Antioxidant Responses of Five Dryland Maize (*Zea mays* L.) Genotypes to Water-Deficit Stress

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Abstract: Maize (*Zea mays*) is a critical staple for global food security, particularly in developing nations facing a projected population increase to 9.7 billion by 2050, necessitating enhanced agricultural productivity and drought-resilient cultivars. This study provides a comprehensive evaluation of the morphological, physiological, and antioxidant responses of five South African dryland maize genotypes (CY1 DKC71-44B, CY2 DKC72-76BR, CY3 DKC74-26R, CW1 DKC75-65BR, and CW3 DKC78-45BRGEN) to drought stress and identifies genotype-specific antioxidant mechanisms associated with drought tolerance. Morphological (shoot growth), physiological (relative water content—RWC), and biochemical (hydrogen peroxide—H₂O₂, lipid peroxidation, ascorbate peroxidase—APX, and superoxide dismutase—SOD activity) parameters were assessed to identify drought tolerance mechanisms. Drought stress significantly reduced shoot length (28–41%), fresh biomass (up to 41%), and relative water content (19–22%) across the evaluated maize genotypes. However, CY3 (DKC74-26R) and CW3 (DKC78-45BRGEN) maintained higher tissue hydration, suggesting superior drought adaptation. Water deficit also induced oxidative stress, resulting in increases in hydrogen peroxide and lipid peroxidation of up to 245%. In response, APX and SOD isozyme activities increased under drought stress, as demonstrated by enhanced in-gel activity and pixel intensity values. Native-PAGE analysis further revealed differential induction of APX and SOD isoforms, indicating genotype-specific antioxidant responses to drought stress. This study provides critical insights for breeding drought-resilient maize varieties for South Africa's climate, improving agricultural productivity despite growing climatic variability.

Keywords: drought-tolerance; oxidative stress; reactive oxygen species; antioxidant enzymes; maize hybrids; relative water content

1. Introduction

Abiotic stress profoundly influences agricultural productivity, impairing growth, development, yield, and seed quality (Imtaiz, 2025; Bukhari, 2025; Bradshaw et al., 2022). Major abiotic stressors that adversely affect plant growth and productivity include heavy metal toxicity, drought, extreme temperature conditions, and high soil salinity (Mishra et al., 2023; Mareri et al., 2022). These environmental factors disrupt plant



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physiological processes and can significantly reduce crop yields. Drought, characterized by prolonged periods of below-average rainfall, poses a major threat to global agriculture by reducing productivity, exacerbating food shortages, and heightening global food insecurity (Vurukonda et al., 2016; Iqbal et al., 2020; Balbaa et al., 2022; Singhal et al., 2023). High drought levels negatively impact plants by impairing root water absorption, causing water stress (Nashar et al., 2025). This stress can lead to membrane damage and disruption of essential physiological and biochemical processes within the plant (Mahatthanaphatcharakun & Taratima, 2025; Hasegawa et al., 2000; Munns, 2002). Elevated water-deficit stress, caused by prolonged drought, can induce significant oxidative damage in plants (Cruz de Carvalho, 2008). This phenomenon is a consequence of the augmented production of reactive oxygen species (ROS), including superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH\cdot$), and singlet oxygen (1O_2) (Gueta-Dahan et al., 1997; Grant & Loake, 2000). Excessive ROS accumulation can subsequently inhibit cell division and expansion, ultimately leading to necrosis (Zhu, 2001). To mitigate this, plants possess robust antioxidant defence systems, categorised into enzymatic defence and non-enzymatic defence pathways (Hasanuzzaman et al., 2019). Enzymatic defence systems include enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and glutathione reductase (GR) (Adamu et al., 2018; Hasanuzzaman et al., 2019). While the non-enzymatic defence systems comprise compounds such as ascorbic acid (AsA), glutathione (GSH), and carotenoids (Ren et al., 2021). Together, the enzymic and non-enzymatic defences work synergistically to prevent oxidative damage to lipids, proteins, and DNA (Cannea & Padiglia, 2025).

In South Africa, the major maize (*Zea mays*) production regions, Free State, Mpumalanga, and North-West provinces, are prone to drought and extreme heat occurrences (Simanjuntak et al., 2023; Tsubo and Moeletsi, 2025). Maize is extremely susceptible to drought and heat stress, which significantly impacts its growth and yield potential (Meng et al., 2016). Similar to most plants, maize may exhibit remarkable adaptability to drought stress by altering its biomass distribution (Pandit et al., 2020; Yan et al., 2023). Predominantly during drought events, maize increases the accumulation of root biomass, sometimes to the detriment of the yield components (Yan et al., 2022). Regardless, due to the vulnerability of maize yields to climate variability, there has been a surge in the breeding of drought and heat-stress-tolerant maize varieties (Sheoran et al., 2022). A pivotal objective in plant breeding initiatives involves the identification of superior-performing genotypes under conditions of environmental stress (Luqman et al., 2023; Iqbal et al., 2021; Yadav et al., 2022; Shemi et al., 2021; Shafiq et al., 2021). To identify genotypes that sustain high performance under conditions of water scarcity, numerous investigations, including convergent breeding techniques, have been conducted on *Zea mays* genotypes (Nawaz et al., 2025; Kumdee et al., 2023; Yadav et al., 2022; Luqman et al., 2023). These studies have focused on the evaluation of diverse morphological, physiological, and biochemical attributes (Hayat et al., 2026). Although many studies have evaluated morphological traits, there is limited comparative data on the synergy between biochemical antioxidant responses and biomass partitioning in drought-resistant hybrids grown in South African conditions. This study focuses on understanding the growth and physiological responses of maize to water scarcity, a foundation necessary for developing effective strategies to enhance drought tolerance in this vital crop. This research is crucial as global warming continues to intensify and poses significant challenges to food security for the continually growing global population (Mirzabaev et al., 2023). To effectively identify drought-adaptive traits in the maize plant, five maize cultivars, namely, CY1 DKC71-44B, CY2 DKC72-76BR, CY3 DKC74-26R, CW1 DKC75-65BR, and CW3 DKC78-45BR, were subjected to water stress to evaluate their morphological, physiological, and biochemical responses to drought conditions. The principal aim of this study was to determine the extent of drought-induced oxidative damage through H_2O_2 and MDA accumulation, assess antioxidant defence responses by analysing APX and SOD isozyme activities and identify representative maize genotypes with superior drought tolerance for potential use in breeding programmes.

2. Materials and Methods

2.1. Study Site

The greenhouse study to evaluate the performance of five selected dryland maize cultivars grown under water stress was conducted during the summer growing season 2025 in Mthatha, Eastern Cape, South Africa.

2.2. Plant Materials

Five selected maize varieties, comprising three yellow and two white hybrids, were used in the experiments (Table 1). These yellow maize varieties are produced and used mostly for livestock feeding, while white varieties are largely used for human consumption by rural small-scale farmers in the Eastern Cape province. The maize seeds used in this study were sourced from Grain-South Africa (Grain-SA).

Table 1. Maize varieties evaluated for tolerance to water stress in the greenhouse.

Variety Name	Type	Origin	Grain Colour
CY1 DKC71-44B	Hybrid	Dekalb®	Yellow
CY3 DKC74-26R	Hybrid	Dekalb®	Yellow
CY2 DKC72-76BR	Hybrid	Dekalb®	Yellow
CW3 DKC78-45BRGEN	Hybrid	Dekalb®	White
CW1 DKC75-65BR	Hybrid	Dekalb®	White

2.3. Experimental Designs and Treatments

A pot experiment was conducted in October 2025 in the botany research laboratory at Walter Sisulu University, Mthatha Campus. The experiment comprised two factors as treatments, with the first being the five selected dry-land maize varieties (CY1 DKC71-44B, CY2 DKC72-76BR, CY3 DKC74-26R, CW1 DKC75-65BR, and CW3 DKC78-45BR) and the second factor was the two water application levels, namely 20 mL and 100 mL. The experiment was arranged in a completely randomized design (CRD) in a 5 × 2 factorial arrangement, consisting of five maize genotypes and two watering regimes. Before sowing, the seeds were surface sterilized with 0.35% sodium hypochlorite (NaOCl) for 5 min, then rinsed five times with sterile water, and imbibed for 16 h to speed up germination. At planting, a total of 30 × 6-litre plastic pots were filled with Promix Organic growing medium, and two seeds were planted per pot. Following seedling establishment, individual plants were transferred into separate pots to ensure that each pot contained one plant. For each maize genotype, three independent plants (biological replicates) were maintained under well-watered conditions and three independent plants were maintained under water-deprived conditions. Each individual pot containing one plant was considered an experimental unit. Thus, each genotype × watering treatment combination consisted of three biological replicates.

After sowing 100 mL was applied into the pots at three days intervals until the first vegetative growth stage (V1 stage). At 7 days after germination the drought stress treatments were induced. The irrigation treatments consisted of a well-watered control receiving 100 mL of water every three days and a drought-stress treatment receiving 20 mL of water once every seven days for the duration of the experiment. The control irrigation regime was based on the routine watering schedule used in our greenhouse to maintain healthy seedling growth. The reduced irrigation regime (20 mL week⁻¹) was selected from preliminary greenhouse observations as the minimum water supply capable of inducing sustained drought symptoms while maintaining plant survival until harvest, thereby allowing physiological and biochemical responses to be evaluated. Pots were then kept for germination and development at 25/19 °C Day/night temperature and 16/8 h light/dark cycle, at a photosynthetic photon flux density of 400 μmol photons·m⁻²·s⁻¹.

2.4. Data Collection and Extract Preparation

The experiment was terminated 21 days after sowing. For each genotype × water-treatment combination, the six plants representing the three biological replicates (two plants per replicate pot) were harvested for morphological, physiological, and biochemical analyses. Shoot length (cm) was measured using a graduated ruler, while fresh weight (g) was determined immediately after harvest using an analytical balance. Relative water content (RWC) was determined using fresh leaf tissue as described in Section 2.5.1.

Following the morphological assessments, leaf tissues designated for biochemical analyses were immediately snap-frozen in liquid nitrogen and stored at –80 °C until further analysis. Frozen samples were subsequently used for the preparation of trichloroacetic acid (TCA) extracts for the determination of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA), as well as crude protein extracts for antioxidant enzyme activity (APX and SOD) assays.

2.4.1. Selection of Representative Genotypes for Biochemical Analyses

Following the evaluation of morphological and physiological responses to drought stress, three representative maize genotypes exhibiting contrasting drought-response phenotypes were selected for subsequent biochemical analyses. The selection was based on their combined performance for shoot length, fresh weight, dry weight, and relative water content under drought stress. CY1 (DKC71-44B) exhibited the smallest reduction in shoot length, CY2 (DKC72-76BR) showed intermediate responses across several measured parameters, whereas CW3 (DKC78-45BRGEN) exhibited the greatest reduction in shoot length but maintained relatively higher tissue water status under drought conditions. The selection of these genotypes therefore enabled a comparative evaluation of oxidative stress markers and antioxidant responses among contrasting drought-response patterns.

2.4.2. Preparation of Trichloroacetic Acid (TCA) Extract

The extracts for the determination of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) were prepared according to modified protocols described by Dhindsa et al. (1981) and Velikova et al. (2000). Approximately 100 mg of frozen leaf tissue was homogenized in liquid nitrogen and extracted in five volumes of ice-cold 6% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 13,000× *g* for 15 min at 4 °C, and the resulting supernatant was collected and used immediately for the determination of H₂O₂ and MDA concentrations.

2.4.3. Protein Extraction and Quantification

Total soluble proteins were extracted for antioxidant enzyme analyses following homogenization of frozen leaf tissue in liquid nitrogen. Approximately 200 mg of powdered tissue was homogenized in 400 µL of ice-cold extraction buffer containing 40 mM potassium phosphate buffer (pH 7.4), 1 mM EDTA, and 5% (w/v) polyvinylpyrrolidone (PVPP). The homogenate was centrifuged at 13,000× *g* for 20 min at 4 °C, and the resulting supernatant was collected as the crude enzyme extract. Protein concentration was determined according to the Bradford (1976) method using bovine serum albumin (BSA) as the protein standard. Briefly, 1 µL of enzyme extract was mixed with Bradford reagent and distilled water, incubated at room temperature for 10 min, and absorbance was measured at 595 nm. Protein concentrations were calculated from a BSA standard curve, and all measurements were performed in triplicate.

2.5. Determination of Physiological and Biochemical Parameters

To evaluate the physiological and biochemical responses of the maize genotypes to drought stress, the harvested leaf tissues were subjected to analyses of relative water content (RWC), oxidative stress markers, and antioxidant enzyme activities as described below.

2.5.1. Relative Water Content

The relative water content (RWC) was determined following the method of Barrs and Weatherley (1962) with slight modifications. Fresh leaf samples were collected immediately after harvest, and the fresh weight (FW) was recorded using an analytical balance. The leaves were then immersed in distilled water and incubated in darkness at room temperature for 24 h to obtain full turgidity. After incubation, excess surface moisture was gently removed using absorbent paper before recording the turgid weight (TW). The samples were subsequently oven-dried at 70 °C for 48 h until a constant weight was achieved, and the dry weight (DW) was recorded.

Relative water content was calculated using the following equation:

$$\text{RWC (\%)} = [(FW - DW)/(TW - DW)] \times 100$$

where FW is the fresh weight, TW is the fully turgid weight, and DW is the dry weight of the leaf tissue.

2.5.2. Estimation of Lipid Peroxidation (MDA)

Lipid peroxidation was estimated by quantifying malondialdehyde (MDA), a product of membrane lipid oxidation, following a modified thiobarbituric acid (TBA) assay described by Dhindsa et al. (1981). An aliquot (100 µL) of the supernatant from the TCA extract was mixed with 400 µL of reaction solution containing 20% (w/v) TCA and 0.5% (w/v) thiobarbituric acid (TBA). The reaction mixture was vortexed thoroughly and incubated at 90 °C for 20 min. The reaction was terminated by cooling the samples on ice

for 10 min, followed by centrifugation at $12,000\times g$ for 5 min at 4 °C and absorbance recorded at 532 nm and 600 nm. After subtracting the non-specific absorbance (A_{600} nm), the MDA concentration was determined by its extinction coefficient of $155\text{ mM}^{-1}\text{ cm}^{-1}$ and expressed as nmol g^{-1} of fresh weight.

2.5.3. Hydrogen Peroxide Content

Hydrogen peroxide (H_2O_2) concentration was determined according to the method that was previously described by Velikova et al. (2000) with minor modifications. The supernatants from the TCA extract were further centrifuged at $12,000\times g$ for 10 min. A total of 50 μL of the supernatant from each sample was used to initiate the reaction in a mixture containing 0.5 M potassium iodide (KI) and 20 mM potassium phosphate buffer (K_2HPO_4 ; pH 5.0) to a final reaction volume of 200 μL .

The reaction mixture was incubated at 25 °C for 20 min, after which absorbance was measured at 390 nm. Hydrogen peroxide concentrations were calculated using a standard curve based on the absorbance (A_{390} nm) of H_2O_2 standards and expressed as $\mu\text{mol H}_2\text{O}_2\text{ g}^{-1}$ fresh weight.

2.5.4. Analysis of Antioxidant Enzyme Activities

Antioxidant enzyme activities of ascorbate peroxidase (APX) and superoxide dismutase (SOD) were analysed by native polyacrylamide gel electrophoresis (Native-PAGE) following the methods of Beauchamp and Fridovich (1971) and Seckin et al. (2010), with minor modifications. Protein extracts (80 μg for SOD and 100 μg for APX) were mixed with a non-denaturing loading buffer and separated on 5% stacking and 15% resolving polyacrylamide gels at 4 °C under a constant voltage of 80 V. Electrophoresis was performed using a running buffer containing 25 mM Tris and 192 mM glycine. For APX analysis, the running buffer was supplemented with 2 mM ascorbic acid to maintain enzyme activity throughout electrophoresis. Separation was terminated when the bromophenol blue tracking dye reached the bottom of the resolving gel.

Determination of APX Activity

Following electrophoresis, APX activity was visualized according to the modified method of Seckin et al. (2010). Gels were equilibrated in 50 mM potassium phosphate buffer (pH 7.0) containing 2 mM ascorbic acid, followed by incubation in 50 mM potassium phosphate buffer (pH 7.8). Enzyme activity was visualized by incubating the gels in staining solution containing 28 mM TEMED and 0.5 mM nitro blue tetrazolium (NBT). All staining procedures were performed in darkness with gentle agitation. APX isoforms appeared as colourless bands against a dark blue background.

Determination of SOD Activity

Superoxide dismutase activity was detected using the method of Beauchamp and Fridovich (1971) with minor modifications. Following electrophoresis, gels were incubated in 50 mM potassium phosphate buffer (pH 7.8), followed by staining in the same buffer containing 2.5 mM NBT. The gels were subsequently transferred to staining solution containing 28 mM TEMED and 0.028 mM riboflavin and exposed to light until achromatic enzyme activity bands became visible against a uniformly blue background.

2.6. Data Analysis

Collected data were subjected to a one-way analysis of variance (ANOVA) using GraphPad Prism version 6.03 (GraphPad Software, Inc., Boston, MA, USA, 2013). Post-hoc comparisons among treatment means were conducted using Tukey's HSD test at a significance level of $p \leq 0.05$. A probability level of 5% was considered significant for all tests.

3. Results

3.1. Morphological Response of Five Selected Dry-Land Maize Genotypes to Induced Water Stress Levels

Water deficit significantly reduced shoot growth in all five maize genotypes (Figure 1; $p < 0.05$). Under water-deprived conditions, shoot length decreased from 29.0 to 25.0 cm in CY1 DKC71-44B (13.8%), from 21.0 to 16.0 cm in CY2 DKC72-76BR (23.8%), from 35.0 to 22.5 cm in CY3 DKC74-26R (35.7%), from 33.7 to 26.5 cm in CW1 DKC75-65BR (21.4%), and from 37.7 to 22.5 cm in CW3 DKC78-45BRGEN (40.3%), relative to their respective well-watered controls. Among the genotypes, CY1 DKC71-44B exhibited the smallest reduction in shoot length, whereas CW3 DKC78-45BRGEN exhibited the greatest reduction in shoot length.

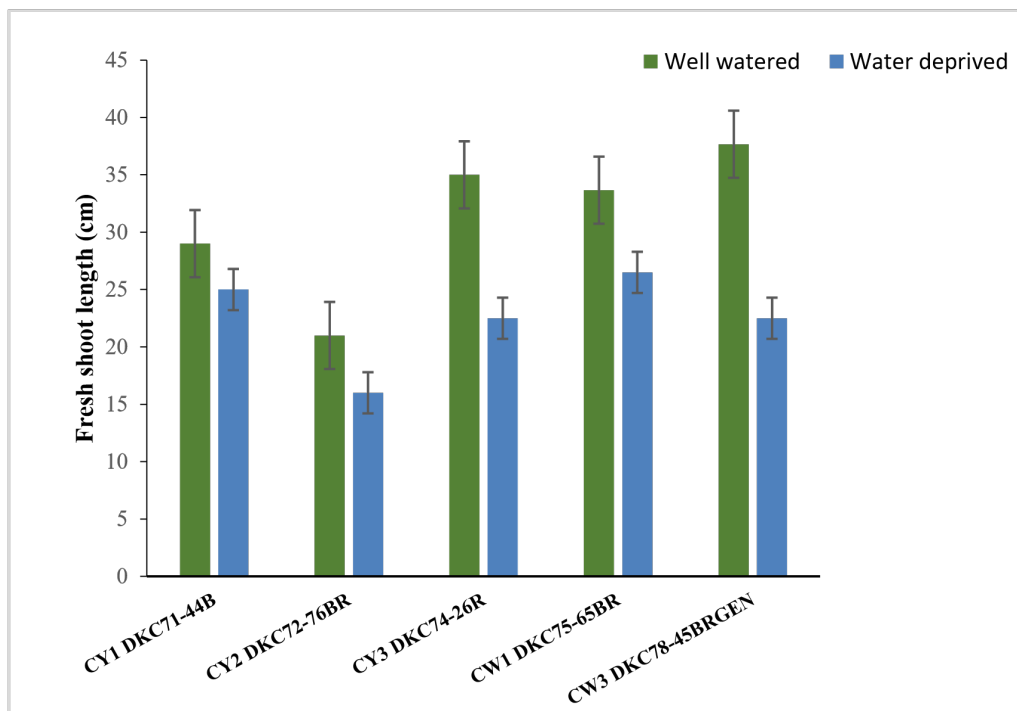


Figure 1. Fresh shoot length of five dryland maize genotypes in response to drought stress. Shoot length, expressed as centimeters (cm), in the leaves of five maize genotypes grown under well-watered (WW; 100 mL) and water-deprived (WD; 20 mL) conditions. Values represent mean $\pm$ SE. Different letters indicate significant differences between treatments ($p \leq 0.05$).

3.2. Biomass Responses of Selected Dryland Maize Genotypes to Water Deficit

As shown in Figure 2, drought stress reduced fresh shoot weight in all five maize genotypes, with reductions of 39.5% in CY1 (1.19–0.72 g), 19.7% in CY2 (0.76–0.61 g), 23.9% in CY3 (1.59–1.21 g), 17.2% in CW1 (1.51–1.25 g), and 22.5% in CW3 (1.60–1.24 g). CY1 (DKC71-44B) recorded the greatest reduction, whereas CW1 (DKC75-65BR) exhibited the smallest reduction in fresh shoot weight.

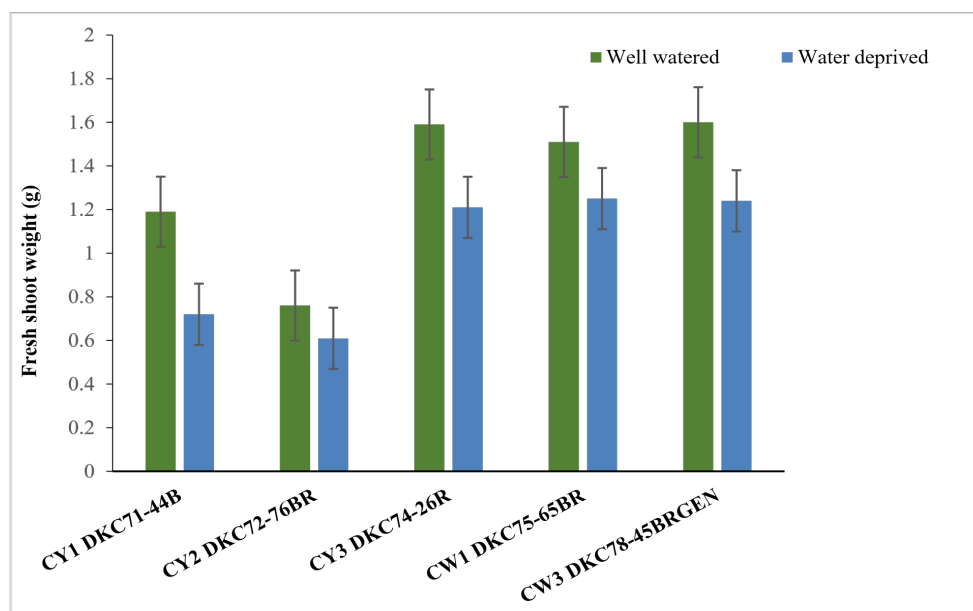


Figure 2. Fresh shoot weight of five dryland maize genotypes response drought stress. Shoot weight, expressed as grams (g), in the leaves of five maize genotypes grown under well-watered (WW; 100 mL) and water-deprived (WD; 20 mL) conditions. Values represent mean $\pm$ SE. Different letters indicate significant differences between treatments ($p \leq 0.05$).

When expressed relative to the well-watered controls, CY1 (DKC71-44B) showed the greatest reduction in fresh shoot weight (39.5%), indicating greater sensitivity to water deficit. In contrast, CW1 (DKC75-65BR) exhibited the smallest reduction (17.2%), while CY2 (DKC72-76BR), CW3 (DKC78-45BRGEN), and CY3 (DKC74-26R) showed intermediate reductions of 19.7%, 22.5%, and 23.9%, respectively. These comparatively smaller reductions suggest a greater capacity to maintain fresh shoot biomass under drought conditions.

Drought treatment produced genotype-dependent effects on dry shoot weight (Figure 3). CY1DKC71-44B showed a reduction in dry shoot weight from 0.15 g under well-watered conditions to 0.10 g under water-deprived conditions, representing a 33.3% decrease. In CY2 (DKC72-76BR), dry shoot weight increased slightly from 0.15 to 0.16 g, whereas CY3 (DKC74-26R) showed a slight decrease from 0.16 to 0.15 g. In contrast, CW1 (DKC75-65BR) showed an increase from 0.11 to 0.19 g, while CW3 (DKC78-45BRGEN) increased from 0.13 to 0.17 g under water-deprived conditions. These results demonstrate genotype-dependent variation in biomass accumulation under water deficit.

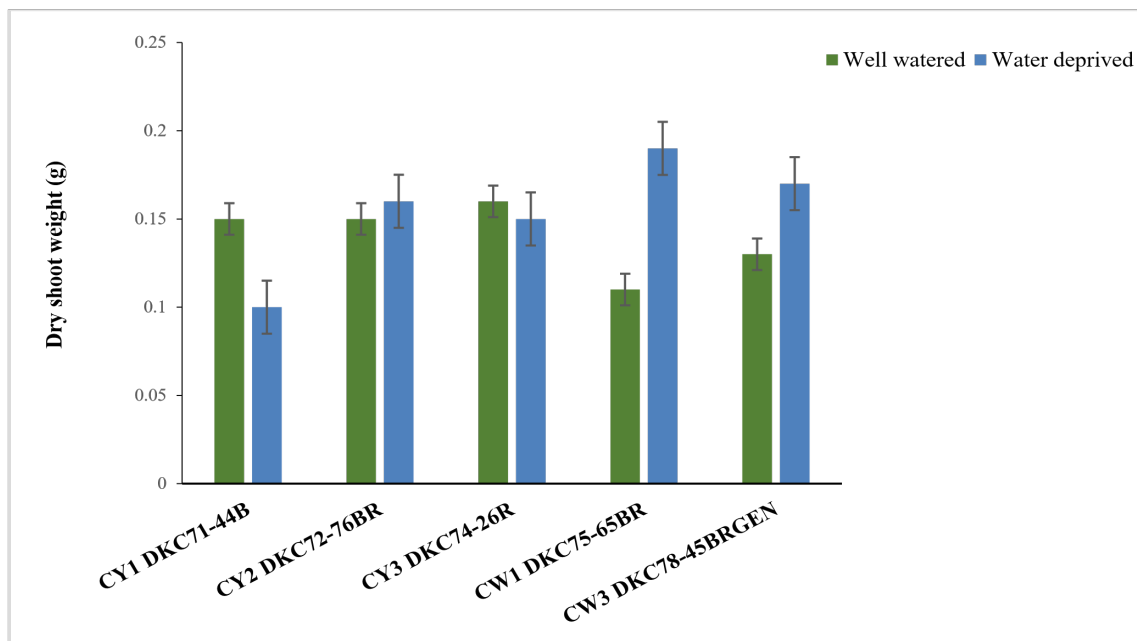


Figure 3. Dry shoot weight of five dryland maize genotypes response drought stress. Shoot dry weight, expressed as grams (g), in the leaves of five maize genotypes grown under well-watered (WW; 100 mL) and water-deprived (WD; 20 mL) conditions. Values represent mean $\pm$ SE. Different letters indicate significant differences between treatments ($p \leq 0.05$).

3.3. Physiological Status and Antioxidant Responses of Selected Maize Genotypes under Drought Stress

To evaluate the level of drought stress induced oxidative stress and the activation of antioxidant defence system associated with drought tolerance, the following parameters were measured.

3.3.1. Relative Water Content (RWC)

As shown in Figure 4, drought stress reduced the relative water content (RWC) of all five maize genotypes when compared with their respective well-watered controls. RWC decreased from 152.90 to 96.91% in CY1 (DKC71-44B), representing a reduction of 36.6%; from 152.50 to 95.45% in CY2 (DKC72-76BR), representing a reduction of 37.4%; from 154.00 to 126.50% in CY3 (DKC74-26R), representing a reduction of 17.9%; from 169.50 to 122.50% in CW1 (DKC75-65BR), representing a reduction of 27.7%; and from 172.00 to 146.00% in CW3 (DKC78-45BRGEN), representing a reduction of 15.1

Although drought stress reduced RWC in all genotypes, the extent of the reduction varied among them, with CY2 (DKC72-76BR) showing the greatest reduction and CW3 (DKC78-45BRGEN) showing the smallest reduction.

The smaller decline in RWC recorded in CW3 (DKC78-45BRGEN) suggests a greater capacity to maintain tissue hydration under water-deficit conditions.

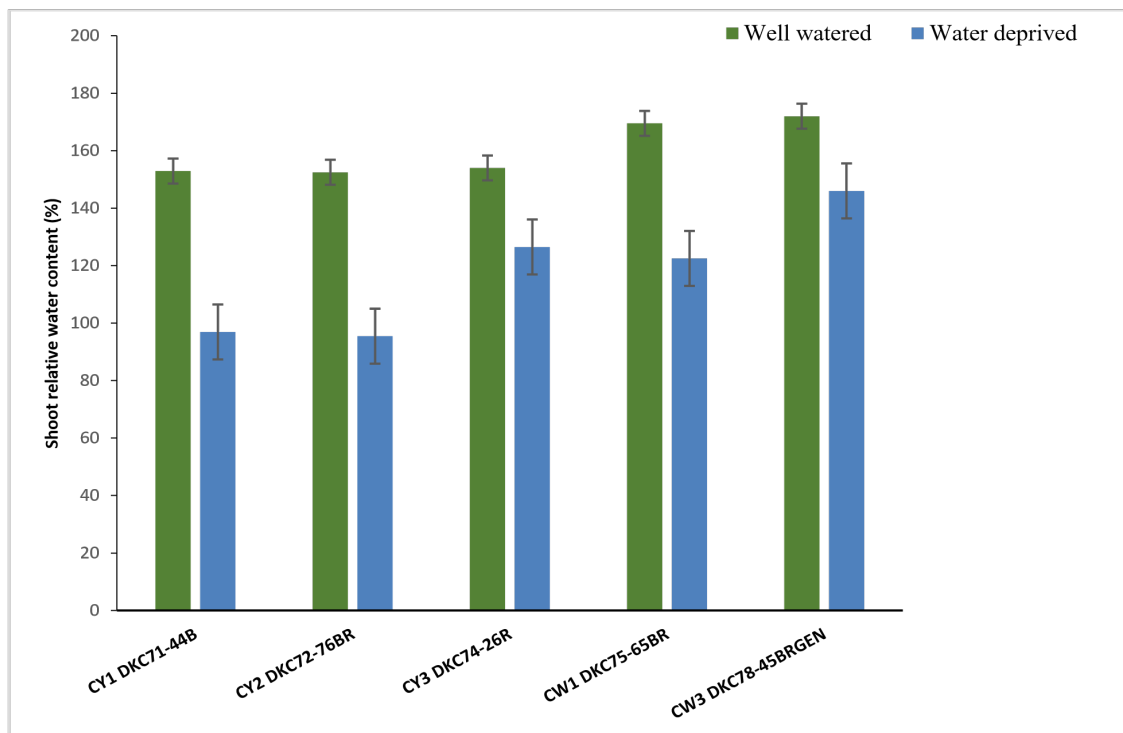


Figure 4. Shoot relative water content (RWC) of five dryland maize genotypes response drought stress. RWC, expressed as percentage (%), in the leaves of five maize genotypes grown under well-watered (WW; 100 mL) and water-deprived (WD; 20 mL) conditions. Values represent mean $\pm$ SE. Different letters indicate significant differences between treatments ($p \leq 0.05$).

3.3.2. Oxidative Stress Response of Selected Maize Genotypes to Induced Water Stress.

Based on the morphological responses to drought stress, three maize genotypes representing contrasting drought responses were selected for subsequent biochemical analyses. These included CY1 (DKC71-44B), which exhibited the greatest drought tolerance, CY2 (DKC72-76BR), which displayed intermediate drought sensitivity, and CW3 (DKC78-45BRGEN), which was identified as the most drought-sensitive genotype. This selection enabled a comparative evaluation of oxidative stress markers and antioxidant responses across contrasting drought-response phenotypes.

Hydrogen Peroxide (H_2O_2) Accumulation under Drought Stress

To further investigate the biochemical mechanisms underlying the contrasting drought responses, hydrogen peroxide (H_2O_2) concentrations were quantified in three representative maize genotypes selected based on their morphological performance under drought stress. These included the drought-tolerant genotype CY1 DKC71-44B, the moderately sensitive genotype CY2 DKC72-76BR and the drought-sensitive genotype CW3 DKC78-45BRGEN.

As shown in Figure 5, drought stress significantly increased H_2O_2 content in all three evaluated genotypes. However, the level of accumulation varied amongst the genotypes.

CY1 (DKC71-44B) exhibited the greatest increase in H_2O_2 content, with a 60% increase relative to the well-watered control. CY2 (DKC72-76BR) and CW3 (DKC78-45BRGEN) also showed significant increases in H_2O_2 content of 43% and 30%, respectively. Although the increases in CY2 and CW3 were statistically significant, they were smaller in magnitude than that observed in CY1. These results indicate genotype-dependent differences in H_2O_2 accumulation under drought stress.

Quantifying Lipid Peroxidation

Drought stress led to increased lipid peroxidation as evidenced by high malondialdehyde (MDA) levels recorded in all maize genotypes when compared with their well-watered controls (Figure 6). The level of MDA accumulation varied amongst genotypes, with CY2 DKC72-76BR exhibiting the greatest increase (245%), followed by CY1 DKC71-44B (195%), whereas CW3 DKC78-45BRGEN showed the smallest increase (134%). This data shows that drought stress increased lipid peroxidation across all genotypes, albeit the extent of oxidative damage varied according to genotype.

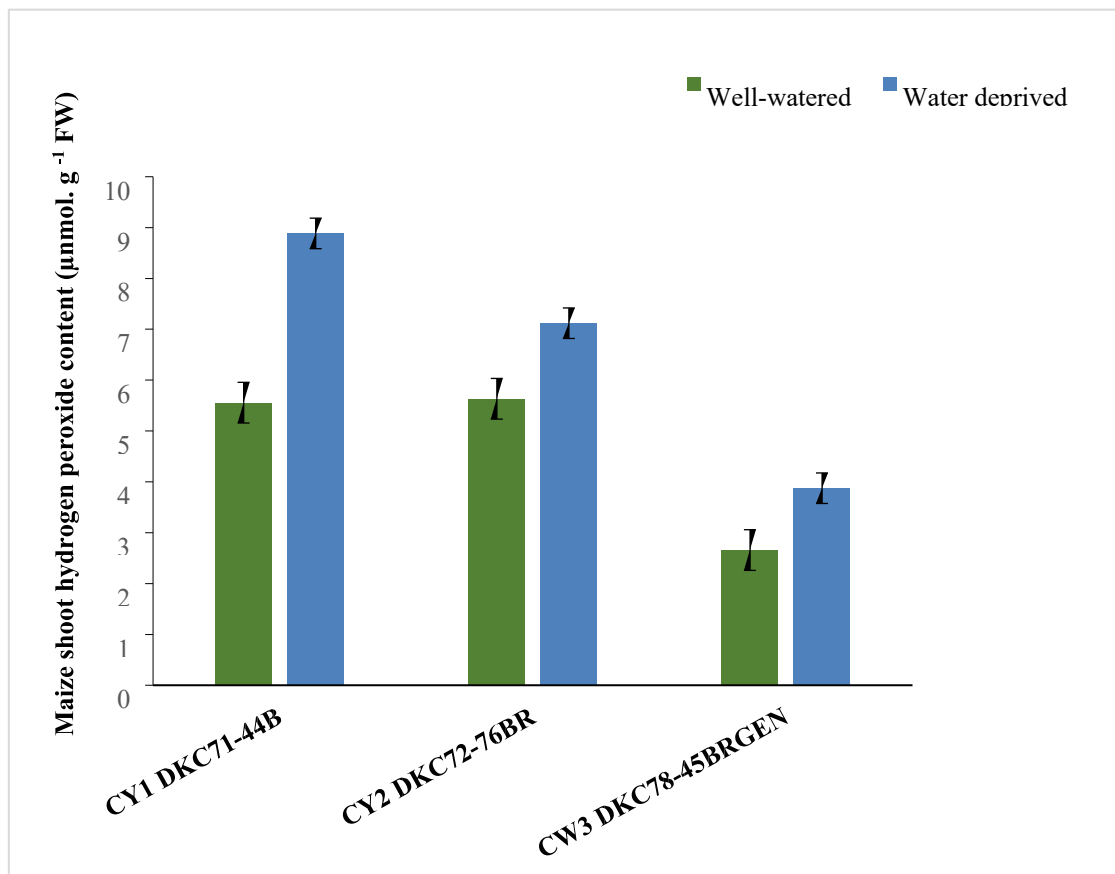


Figure 5. Hydrogen peroxide (H₂O₂) content in the leaves of three selected maize genotypes grown under well-watered (WW; 100 mL) and water-deprived (WD; 20 mL) conditions. Values represent mean ± SE. Different letters indicate significant differences between treatments ($p \leq 0.05$).

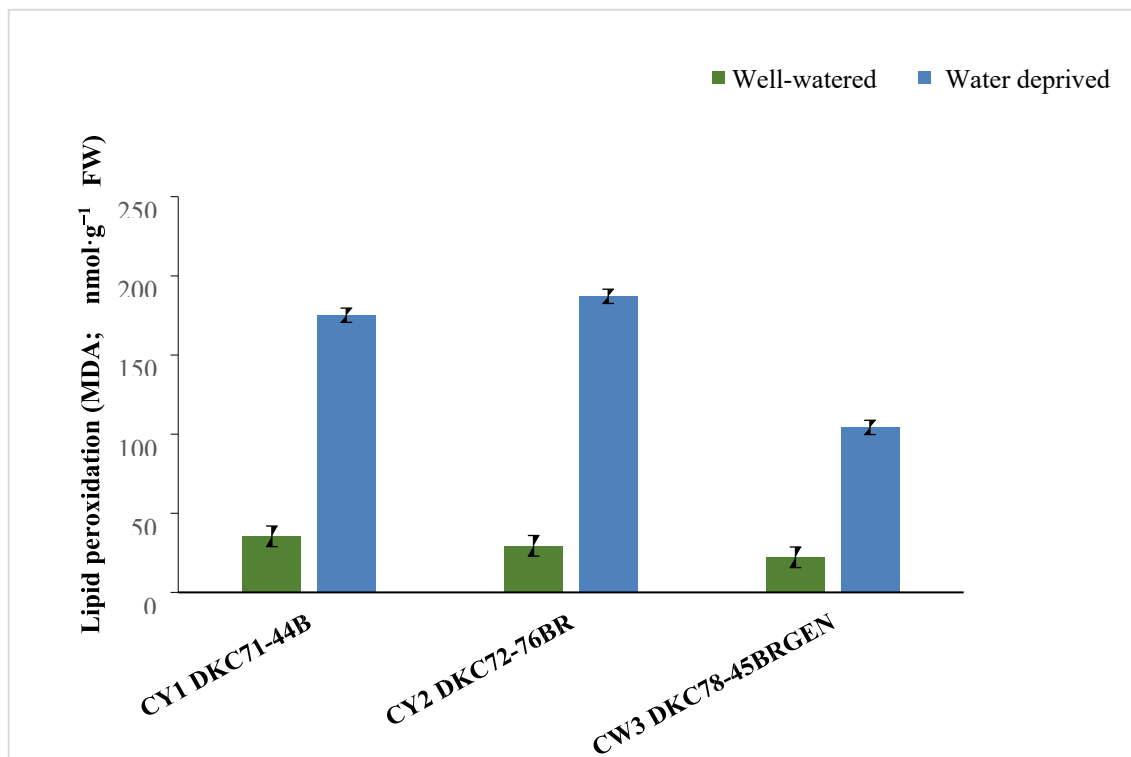


Figure 6. Lipid peroxidation, expressed as malondialdehyde (MDA) concentration, in the leaves of three selected maize genotypes grown under well-watered (WW; 100 mL) and water-deprived (WD; 20 mL) conditions. Values represent mean ± SE. Different letters indicate significant differences between treatments ($p \leq 0.05$).

3.3.3. Antioxidant Enzyme Responses of Selected Maize Genotypes to Drought Stress

The fact that there were a genotype-dependent differences in hydrogen peroxide (H₂O₂) accumulation and lipid peroxidation (MDA content) under drought stress prompted further investigation of the antioxidant defence response. To determine whether these contrasting oxidative stress responses were associated with changes in antioxidant enzyme activity, the activity of individual ascorbate peroxidase (APX) and superoxide dismutase (SOD) isoforms were evaluated using native in-gel activity assays coupled with pixel intensity analysis.

Ascorbate Peroxidase (APX) Isozyme Activity

As shown in Figure 7, native in-gel electrophoresis revealed four distinct APX isoforms, designated Maize APX1, APX2, APX3, and APX4 respectively based on their relative migratory rates

All four isoforms were detected in the shoots of the three representative maize genotypes under both well-watered and drought conditions. Although the same APX isoforms were expressed across all genotypes, differences in band intensity were evident between treatments, indicating that drought stress altered APX activity in a genotype-dependent manner. Visual differences in band intensity were subsequently evaluated using pixel densitometric analysis.

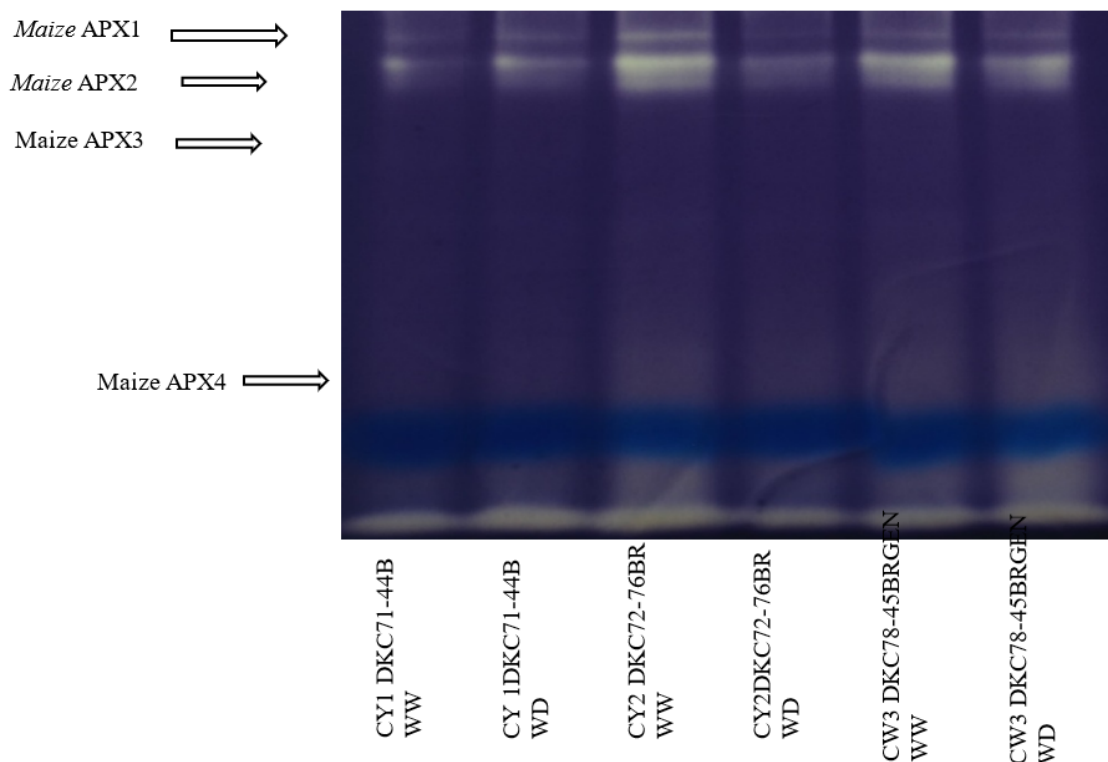


Figure 7. Native-PAGE profiles of ascorbate peroxidase (APX) isoforms in the shoots of three representative maize genotypes grown under well-watered (WW) and water-deprived (WD) conditions.

As shown in Figure 8, pixel densitometric analysis indicated that drought stress increased the activity of all four APX isoforms in each of the three representative maize genotypes. In CY1 (DKC71-44B), APX1, APX2, APX3, and APX4 increased from approximately 23, 30, 26, and 31 arbitrary units under well-watered conditions to 30, 35, 32, and 38 arbitrary units, respectively, under drought stress. In CY2 (DKC72-76BR), the corresponding values increased from approximately 23, 31, 30, and 29 arbitrary units to 27, 36, 37, and 36 arbitrary units, respectively. Similarly, in CW3 (DKC78-48BRGEN), APX1 increased from approximately 27 to 31 arbitrary units, APX2 from 31 to 40 arbitrary units, APX3 from 33 to 38 arbitrary units, and APX4 from 29 to 36 arbitrary units.

The relative intensity of individual APX isoforms varied among genotypes under drought stress. APX4 exhibited the highest pixel intensity in CY1, APX3 exhibited the highest intensity in CY2, and APX2 exhibited the highest intensity in CW3. APX1 showed the lowest pixel intensity under drought stress across all three genotypes. Overall, the results demonstrate genotype-dependent and isoform-specific differences in APX

activity in response to drought stress. These findings suggest differential activation of APX isoforms as part of the antioxidant response to drought-induced oxidative stress.

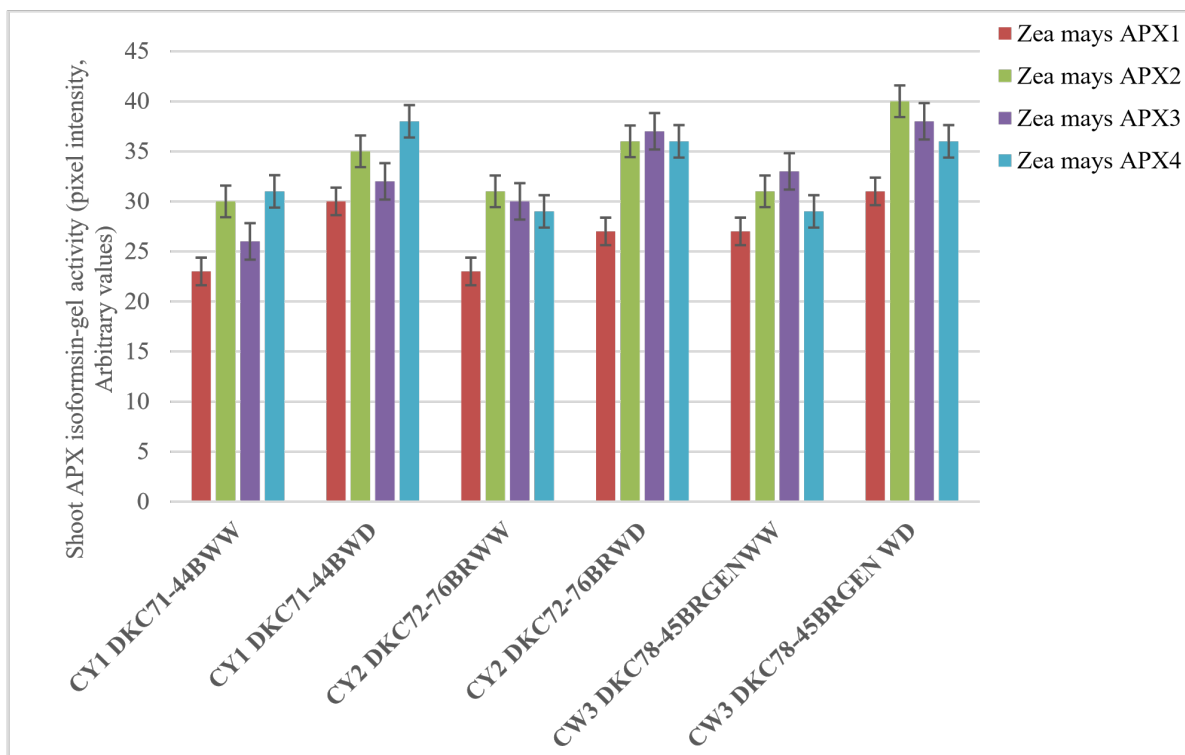


Figure 8. Pixel intensity values signifying the level of APX activity derived from analysis of the band intensity of three selected maize genotypes following 21 days of growth under well-watered (WW) and water-deprived (WD) conditions. Values represent mean $\pm$ SE. Different letters indicate significant differences ($p \leq 0.05$).

Effect of water deprivation on activity and expression profile of superoxide dismutase (SOD) isozymes

As shown in Figure 9, visual analysis of the native PAGE gel showed the presence of five SOD isoforms, named as *Zea mays* SOD1, SOD2, SOD3, SOD4, and SOD5 respectively based on their relative migratory rate. All five isoforms were detected in the shoots of the three selected maize genotypes (CY1 DKC71-44B, CY2 DKC72-76BR, CW3 DKC78-45BRGEN) under both well-watered and drought conditions. However, there were marked differences in band intensity between treatments, indicating that drought stress influenced SOD isozyme activity in a genotype-dependent manner.

Among the detected isoforms, SOD5 showed the highest band intensity across all three genotypes, whereas SOD1 consistently had the least intensity which reflects their level of activity. The intermediate isoforms (SOD2–SOD4) showed moderate activity under both watering regimes, with noticeably stronger band intensity in drought-treated plants. Overall, the increased intensity of the SOD activity bands under drought conditions suggests enhanced activation of the superoxide scavenging system in response to oxidative stress.

Further analysis using the pixel densitometric evaluation confirmed the visual assessment of the native SOD isozyme profiles (Figure 10). Drought stress increased the activity of all five SOD isoforms in the three selected maize genotypes, although the magnitude of induction varied among individual isoforms.

In CY1 DKC71-44B, SOD isoform activities increased from approximately 23, 31, 26, 30, and 43 arbitrary units under well-watered conditions to approximately 31, 36, 32, 38, and 48 arbitrary units, respectively, following drought treatment. A similar trend was observed in CY2 DKC72-76BR which showed an increased activity of all SOD isoforms, with pixel intensity values increasing from approximately 24, 33, 30, 32, and 33 arbitrary units under well-watered conditions to 29, 38, 41, 36, and 45 arbitrary units, respectively. Likewise, in CW3 DKC78-45BRGEN, a similar trend was observed, where drought stress increased SOD1 from approximately 27 to 31 arbitrary units, SOD2 from 31 to 40 arbitrary units, SOD3 from 34 to 37 arbitrary units, SOD4 from 29 to 36 arbitrary units, and SOD5 from 42 to 49 arbitrary units.

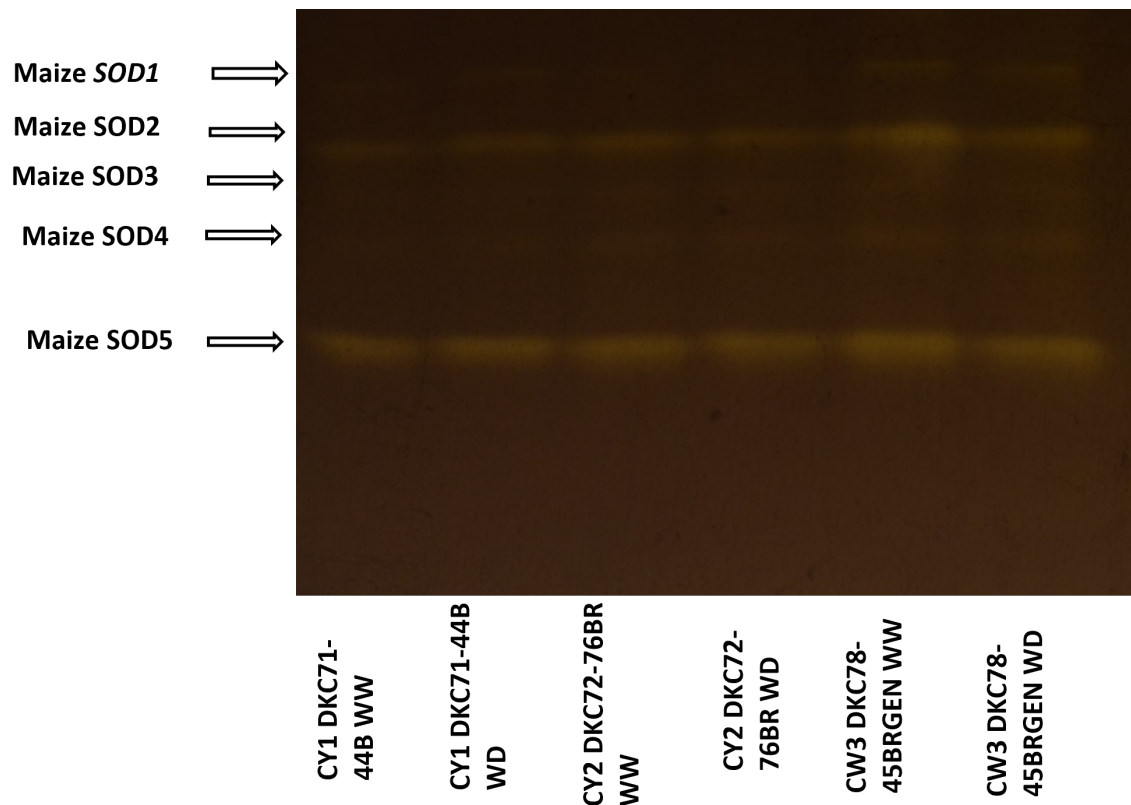


Figure 9. Native-PAGE profiles of superoxide dismutase (SOD) isoforms in shoots of three representative maize genotypes grown under well-watered (WW) and water-deprived (WD) conditions. Five SOD isoforms (SOD1–SOD5) were identified based on their relative electrophoretic mobility.

Among the five isoforms, SOD5 consistently exhibited the highest pixel intensity under drought stress, whereas SOD1 displayed the lowest activity across all three genotypes. Furthermore, the enhanced expression of SOD isoforms under water-deficit conditions suggests an activation of the antioxidant defence system to facilitate the detoxification of superoxide radicals generated during drought stress.

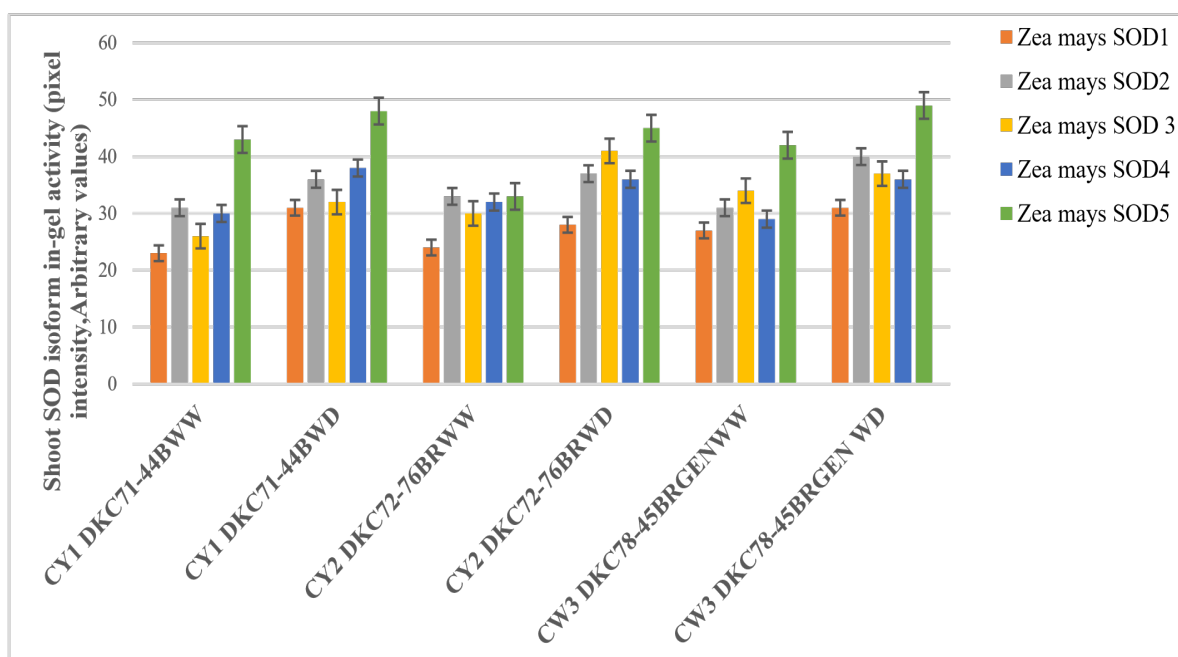


Figure 10. Relative SOD isozyme activity determined by pixel intensity analysis in shoots of three representative maize genotypes grown under well-watered (WW) and water-deprived (WD) conditions. Values represent mean $\pm$ SE. Different letters indicate significant differences between treatments ($p \leq 0.05$).

4. Discussion

Maize (*Zea mays*) is a widely grown staple food crop in most parts of the world due to its adaptability and productivity (Dumani et al., 2024). The crop thrives across different soil types and climatic conditions (Farid et al., 2025). However, drought stress significantly impacts maize growth and yield (Hussain et al., 2019). Water scarcity adversely affects crop physiology and biochemistry, disrupting the life cycle (Ali et al., 2013; Khan et al., 2019). Maize, particularly vulnerable during seedling emergence, exhibits high sensitivity to drought (Ali & Ludidi, 2021). However, substantial genotypic variability in drought response exists within maize, as demonstrated by numerous studies (Doğru & Ecem Bayram, 2016; Hasan et al., 2023). To further explore this variability, particularly at the biochemical level, this study subjected three maize cultivars to a 21-day drought stress period. Subsequent comparative biochemical analyses aimed to elucidate differential tolerance mechanisms among these genotypes under water-limited conditions.

The response of plants to water deprivation is highly variable, influenced by plant species, developmental stage, drought duration, and intensity (Kumdee et al., 2023). The imposition of water deprivation in this study significantly impacted the biomass accumulation of both shoot and root systems across five evaluated maize cultivars. Specifically, a reduction in fresh shoot length was a consistent response to water deficit (WD) in all tested varieties (CY1 DKC71-44B, CY2 DKC72-76BR, CY3 DKC74-26R, CW1 DKC75-65BR, CW3 DKC78-45BRGEN) when compared to their respective well-watered (WW) controls. Notably, the cultivar CY1 DKC71-44B under WD conditions demonstrated comparatively higher tolerance, while CY2 DKC72-76BR WD exhibited the lowest tolerance based on this specific growth parameter. These findings are corroborated by existing literature indicating a negative impact of increasing drought stress on maize growth characteristics, notably shoot length (Abu-Ria et al., 2024). Analogous growth reductions have also been documented in water-stressed maize grown in pot systems (Ahmad et al., 2022) and in sorghum seedlings under field conditions experiencing water deficit (de Queiroz et al., 2023). The observed reductions in plant growth under drought conditions are primarily a consequence of impaired water uptake, which subsequently induces cellular dehydration and the suppression of fundamental developmental processes, including cell expansion, proliferation, and division (Wahab et al., 2022).

Fresh shoot weight was significantly reduced in all water-deprived maize varieties compared to their well-watered counterparts. These results were consistent with findings by Abu-Ria et al. (2024). Regarding dry shoot weight, CY2 DKC72-76BR and CY3 DKC74-26R showed no significant differences between treatments, while CW1 DKC75-65BR and CW3 DKC78-45BRGEN exhibited increased dry shoot weight under water deprivation.

This may reflect drought tolerance mechanisms involving lower stomatal conductance and transpiration rates, as seen in soybean (Sintaha et al., 2022). Conversely, CY1 DKC71-44B showed a decrease in dry shoot weight under drought, aligning with studies in drought-sensitive wheat (Hameed et al., 2013) and maize (Talaat et al., 2015).

From the present study, the maintenance or slight increase in shoot dry weight in some genotypes under drought stress may point to a possible biomass accumulation irrespective of the water-deficit stress. This ability may be associated with improved drought adaptation through osmotic adjustment, maintenance of structural biomass, altered assimilate allocation, and reduced tissue water content.

Relative water content (RWC) is a key metric for assessing plant tissue metabolic activity and water status, reflecting the balance between water supply and transpiration (Soltys-Kalina et al., 2016). In the present study, a significant decline in leaf RWC was observed in all water stressed maize cultivars in comparison to well-watered controls. The magnitude of this reduction was genotype dependent as observed with CY3 DKC74-26R and CW3 DKC78-45BRGEN maintaining relatively higher RWC under drought stress than the other genotypes. This reduction aligns with findings in drought-stressed wheat (Khazaei & Estaji, 2020) and sorghum (Abu-Ria et al., 2024), where water limitations disrupted water balance, leading to decreased RWC. However, contrasting results have been reported in other plant species (Ding et al., 2012; Ding et al., 2014; Nguyen et al., 2020), where RWC increased under drought. The observed elevation is due to enhanced stomatal regulation and an increased root-to-shoot ratio, which facilitates improved water conservation (Wolf et al., 2016; Kou et al., 2022).

The ability to maintain higher RWC under drought conditions is considered an important adaptive trait because it enables plants to sustain cell turgor, preserve stomatal function, and maintain essential physiological processes such as photosynthesis, nutrient transport, and metabolic activity. As such, the higher RWC in CY3 DKC74-26R and CW3 DKC78-45BRGEN may therefore have contributed to the comparatively smaller reductions in shoot growth and biomass observed in these genotypes under drought

stress. Furthermore, the higher RWC maintained by CW3 DKC78-45BRGEN corresponded with lower oxidative damage and enhanced antioxidant enzyme responses, suggesting that maintenance of tissue hydration may be closely associated with improved drought stress tolerance.

Drought stress impairs plant productivity by disrupting physiological homeostasis, primarily through overaccumulation of reactive oxygen species (ROS) that overwhelm cellular antioxidant systems (Bao et al., 2024). This oxidative imbalance induces lipid peroxidation in cellular membranes, as evidenced by elevated malondialdehyde (MDA) levels in drought-stressed plants (Choudhury et al., 2022; Ali and Ludidi, 2021). Concurrently, ROS accelerate leaf senescence through chlorophyll degradation and thylakoid membrane disassembly, a survival strategy that prioritizes nutrient remobilization to younger tissues (Lee & Park, 2012). An elevation in the level of hydrogen peroxide (H_2O_2) was detected in water stressed cultivars (CY1 DKC71-44B, CY2 DKC72-76BR, CW3 DKC78-45BRGEN) relative to well-watered controls, corroborating earlier reports of increased H_2O_2 and malondialdehyde (MDA) accumulation in drought-stressed maize leaves (Ali and Ludidi, 2021). Though historically labeled a damaging reactive oxygen species (ROS), studies emphasize H_2O_2 's pivotal role as a redox signal governing diverse biological functions, from stress adaptation to growth regulation (Asgher et al., 2021; Nazir et al., 2020). As a stress-responsive messenger, H_2O_2 activates pathways that bolster photosynthetic capacity and biomass production under abiotic challenges, including drought (Asgher et al., 2021; Sun et al., 2016; Hung et al., 2005; Neill et al., 2002). Notably, externally applied H_2O_2 can amplify drought resilience in maize by upregulating antioxidant defences—such as superoxide dismutase (SOD) and catalase (CAT)—while curtailing lipid peroxidation and enhancing osmoprotectant synthesis (Demiralay et al., 2022). These insights reaffirm H_2O_2 's dual function: it orchestrates stress-adaptive signaling while simultaneously fine-tuning antioxidant systems to alleviate oxidative harm during water scarcity (Gupta et al., 2016; Abd Elhady et al., 2021; Asgher et al., 2021).

Oxidative stress, whether induced by environmental factors or senescence, triggers lipid peroxidation in plants, as noted by Zhang et al. (2021). Increased MDA content is a key indicator of an increased ROS in the cell. This process disrupts the plant's physiology by compromising membrane integrity, leading to protein denaturation and impairing essential metabolic processes such as photosynthesis and respiration, ultimately leading to cell death under this condition. In this study, a significant increase in lipid peroxidation, a marker of oxidative damage, was observed across all drought-stressed maize. It is important to note at this point that the differential degree of lipid peroxidation amongst the three selected genotypes suggests that membrane stability under drought stress is genotype dependent. Our study suggests that this may indicate a greater capacity to limit oxidative membrane damage, potentially through more effective antioxidant defence mechanisms. As such, our data suggests that lipid peroxidation is not just an indicator of drought-induced injury but also an indicator of the level of effectiveness of the antioxidant system within each genotype.

This finding from the present study supports the results of Nawaz and Wang (2020), who reported elevated lipid peroxidation in *Axonopus compressus* concurrent with increased hydrogen peroxide and malondialdehyde (MDA) levels under drought conditions. Conversely, Kusvuran (2021) found a reduction in lipid peroxidation in drought-stressed broccoli, a response attributed to the protective effects of foliar microalgae application.

Reactive oxygen species (ROS) function as key signaling regulators in plant responses to both abiotic and biotic stresses, dynamically mediating the integration of environmental stimuli with cellular adaptation mechanisms (Mittler et al., 2022; Sewelam et al., 2016; Pitzschke et al., 2006). Beyond their established role in inducing oxidative damage, specific ROS, including hydrogen peroxide (H_2O_2), superoxide radicals ($O_2^{\cdot-}$), and hydroxyl radicals ($OH^{\cdot}$), are involved in the regulation of essential developmental processes, such as the modulation of root architecture, the control of stomatal aperture, and the orchestration of programmed cell death (Mittler et al., 2022; Sewelam et al., 2016; Pitzschke et al., 2006). For example, NADPH oxidases (e.g., *Arabidopsis* RbohD and RbohF) facilitate the generation of apoplastic ROS, which initiate systemic signaling cascades in response to abiotic stresses like heat and salinity, and biotic stresses such as pathogen attack. These ROS signals also engage in crosstalk with phytohormones, including abscisic acid (ABA), to precisely mediate plant stress responses (Mittler et al., 2022; Sewelam et al., 2016).

Reactive oxygen species (ROS), functioning both as signaling molecules and cytotoxic agents, are tightly regulated at cellular and subcellular levels to maintain redox homeostasis while preserving their essential functions in stress signaling and development (Bao et al., 2024; Wang et al., 2024; Meyer et al., 2021; Mittler et al., 2022). In addition to structural defences like lignified cell walls and cuticles (Vrancken et al., 2013), plants employ a multilayered antioxidant system comprising enzymatic and non-enzymatic components to neutralize excess ROS. This system includes primary antioxidant enzymes such as

superoxide dismutase (SOD), which dismutates O_2^- to H_2O_2 ; catalase (CAT), which degrades H_2O_2 to H_2O and O_2 ; and ascorbate peroxidase (APX), a key enzyme in the ascorbate-glutathione cycle that scavenges H_2O_2 (Bao et al., 2024; Abdulfatah, 2022; Meyer et al., 2021). Secondary antioxidants, including glutathione reductase (GR), peroxiredoxins (Prx), and thioredoxins (Trx), regenerate redox buffers and repair oxidative damage (Meyer et al., 2021). Non-enzymatic antioxidants like ascorbate, glutathione, flavonoids, and carotenoids directly quench ROS or stabilize membrane integrity (Bao et al., 2024; Abdulfatah, 2022; Meyer et al., 2021).

Ascorbate peroxidase (APX) plays a vital role in plant drought tolerance by detoxifying hydrogen peroxide (H_2O_2) and scavenging free radicals (Arshadi-Bidgoli et al., 2025; Haghpahan et al., 2024). In this study, water-stressed maize varieties (CY1 DKC71-44B, CY2 DKC72-76BR, and CW3 DKC78-45BRGEN) exhibited a marked increase in APX activity relative to their well-watered counterparts. This increase in enzyme activity corresponded with higher levels of H_2O_2 and lipid peroxidation observed under drought conditions. These results concur with the findings by Ali and Ludidi (2021), suggesting that an increased APX activity mitigates oxidative damage from excess H_2O_2 .

Furthermore, in addition to the overall increase in APX activity under drought stress, an important observation in the present study was the genotype-dependent variation in the activity of individual APX isoforms. Although drought stress increased the activity of all four detected APX isoforms in the three representative maize genotypes, the isoform exhibiting the greatest activity differed among genotypes. APX4 showed the highest activity in CY1 (DKC71-44B), APX3 showed the highest activity in CY2 (DKC72-76BR), whereas APX2 exhibited the highest activity in CW3 (DKC78-45BRGEN). In contrast, APX1 consistently showed the lowest activity under drought conditions across the three genotypes.

These findings indicate that the antioxidant response to drought was both genotype- and isoform-dependent. Rather than a single APX isoform being uniformly dominant across all genotypes, the observed variation suggests that different genotypes may preferentially activate different APX isoforms in response to water deficit. Such differential activation may contribute to the regulation of hydrogen peroxide homeostasis and the mitigation of oxidative stress under drought conditions. The pronounced activity of APX2, APX3 and APX4 suggests that it is possible these isoforms may represent the major H_2O_2 -scavenging enzyme during drought stress, while the relatively constant activity of APX1 may indicate a constitutively expressed housekeeping antioxidant involved in maintaining basal cellular redox homeostasis.

Superoxide dismutase (SOD) is a critical antioxidant enzyme that catalyzes the dismutation of superoxide anions (O_2^-) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), thereby providing the first line of defence against reactive oxygen species (ROS)-induced cellular damage (Azarabadi et al., 2017). SOD also protects membrane integrity by eliminating superoxide radicals and reducing lipid peroxidation (Aslam et al., 2017). This study found increased SOD activity in the shoots of water-deprived maize varieties, likely a response to elevated superoxide anion levels under drought stress, further supported by Ali and Ludidi (2021).

Furthermore, the present study showed that drought-induced stress induces five SOD isoforms although the degree of expression differed amongst individual isoforms. However, only two isoforms were pronounced under drought stress: *Zea mays* SOD1 and SOD5. SOD5 consistently showed the greatest activity under drought stress, whereas SOD1 showed comparatively lower activity across all three representative genotypes. Our study suggests that since SOD catalyses the first line of defence in detoxifying ROS superoxide radicals into hydrogen peroxide, the enhanced expression of SOD5 may represent a primary protective response that limits oxidative damage during the early stages of drought stress.

Our results show that SOD and APX play a crucial role as components of the antioxidant defence system mobilized during drought stress. While SOD, most likely *Zea mays* SOD5 isoforms, are induced in the rapid detoxification of superoxide radicals into hydrogen peroxide, this is followed by APX-mediated scavenging of the hydrogen peroxide into water and oxygen. This coordinated regulation is genotype specific and is essential for maintaining cellular redox homeostasis, protecting membrane integrity, and sustaining metabolic activity during prolonged water limitation.

Our strategy to assess various parameters such as morphological traits, relative water content, oxidative stress markers, and antioxidant enzyme activities has indeed demonstrated that drought tolerance in maize is a complex trait involving coordinated physiological and biochemical responses. As such, this study encourages plant breeders to cultivate genotypes that can maintain tissue hydration and at the same time be able to efficiently mobilize antioxidant defence system during drought stress. This coordinated attribute can serve as an important physiological biomarker for screening maize germplasm for improved drought tolerance in breeding programmes most especially in drought prone areas.

5. Conclusions

Ensuring food security under climate change requires the identification and development of maize (*Zea mays*) genotypes with improved resilience to water-limited conditions. The present study demonstrated that drought stress adversely affected early maize growth and water relations, as reflected by reductions in shoot growth, fresh biomass accumulation, and relative water content (RWC). Drought stress was also associated with increased hydrogen peroxide (H₂O₂) accumulation and malondialdehyde (MDA) levels, indicating enhanced oxidative stress and lipid peroxidation. These responses demonstrate that water deficit disrupts cellular water balance and induces oxidative damage during the maize seedling stage.

The five evaluated maize genotypes exhibited distinct physiological and biochemical responses to drought stress, indicating considerable genotypic variation in their ability to cope with water deficit. Increased responses of the antioxidant enzymes ascorbate peroxidase (APX) and superoxide dismutase (SOD) under drought suggest that enzymatic antioxidant defence contributes to the mitigation of drought-induced oxidative stress. However, the magnitude of these responses varied among genotypes, highlighting differences in their physiological and biochemical adjustment to water deficit.

Among the evaluated genotypes, CY1 (DKC71-44B) showed a comparatively strong antioxidant response, although it also experienced the greatest reduction in fresh shoot biomass under drought stress. CY2 (DKC72-76BR) exhibited the greatest reduction in RWC, suggesting greater impairment of plant water status under the imposed stress conditions. In contrast, CW3 (DKC78-45BRGEN) maintained the smallest reduction in RWC, indicating comparatively better maintenance of water status under drought. These contrasting responses demonstrate that drought adaptation cannot be inferred from a single physiological or biochemical trait but rather involves the coordinated regulation of growth, water status, oxidative stress, and antioxidant defence.

The findings provide useful insights into the physiological and biochemical responses of the evaluated maize genotypes to water deficit and may contribute to the identification of drought-responsive traits relevant to maize improvement in South Africa. In particular, the observed variation in biomass production, water status, oxidative stress markers, and antioxidant responses provides a basis for further evaluation of these genotypes under more controlled and field-relevant drought conditions. Nevertheless, the present findings should be interpreted within the context of the seedling-stage greenhouse experiment and should not be taken as definitive evidence of field-level drought tolerance.

A limitation of the present study is that drought stress was imposed by simultaneously reducing irrigation volume and increasing the interval between watering events. Although this approach successfully induced water deficit under greenhouse conditions, it did not allow the independent effects of irrigation volume and watering frequency to be distinguished. Future studies should therefore impose drought stress using a standardized soil-water regime, such as defined soil water content or field-capacity-based irrigation, while maintaining a consistent irrigation interval. Additional experiments extending beyond the seedling stage and incorporating field conditions, yield-related traits, and larger numbers of genotypes would further strengthen the assessment of drought tolerance.

Overall, the coordinated responses of growth, water status, oxidative stress markers, and antioxidant enzymes demonstrate that maize responses to drought involve an integrated physiological and biochemical adjustment. The contrasting responses observed among the evaluated genotypes provide a useful foundation for further investigation and validation of drought-responsive traits that may ultimately support maize breeding and cultivar selection for water-limited environments.

Author Contributions

Y.S.G.: Conceptualization, Writing—Initial Draft, Writing, Revisions, Data Curation, Investigation, Visualization; A.D.: Writing, Review and Editing, Data Curation; N.L.: Conceptualization, Writing, Review and Editing, Supervision; I.M.E.: Conceptualization, Resources, Review and Editing, Supervision. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

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Data Availability Statement

All data generated in this study are included in this article for publication.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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