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Evaluation of Morphological and Physiological Traits of Maize Genotypes under Drought Stress Conditions

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ABSTRACT

The purpose of the research was to determine drought-tolerant genotypes, as well as examine and contrast their responses under normal and severe drought conditions. The investigation was applied in a factorial form based on a Randomised Complete Design (RCD) with seven maize genotypes (KSC201, KSC704, KSC705, KSC706, KSC7158, KSC647, and TWC647) and drought stress at three levels (0, 2, 4, and 8 bar) in three replicates. The study revealed that the most plant height, total dry weight, and root dry weight (33.64, 2.29, and 0.83 g plant^{-1}) were recorded in genotype KSC7158 in no-plants stress, respectively. A severe drought reduced the amount of chlorophyll and relative water content. The cultivar of KSC7158 had the most total chlorophyll and relative water content. The crop's total soluble sugar and proline concentration were increased in 8-bar conditions. The KSC647 genotype had the highest proline content, whereas the KC7158 genotype had the highest total soluble sugar. The reactions of catalase, ascorbate peroxidase, and polyphenol oxidase increased in 8-bar conditions, with the genotypes of KSC7158 and KSC706 showing the highest enzyme activity. Furthermore, KSC7158, KSC706, and KSC201 hybrids exhibited superior growth parameters under both normal and severe drought conditions, in comparison to other hybrids. In terms of every trait examined under severe drought, the KSC7158 hybrid was found to be tolerant.

Field Crops

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Kuraklık Stresi Şartlarında Mısır Genotiplerinin Morfolojik ve Fizyolojik Özelliklerinin Değerlendirilmesi

ÖZET

Bu çalışma, normal koşullar ve kuraklık stresi altında mısır genotiplerinin tepkilerini değerlendirmek ve kuraklığa dayanıklı genotipleri karşılaştırmalı olarak belirlemek amacıyla gerçekleştirilmiştir. Deneme, yedi mısır genotipi (KSC201, KSC704, KSC705, KSC706, KSC7158, KSC647, TWC647) ve üç seviyede (0, 2, 4 ve 8 bar) kuraklık stresi ile üç tekrarlamalı olarak tesadüf blokları deneme desenine dayalı faktöriyel düzende yürütülmüştür. Araştırma sonucunda, bitki boyu, toplam kuru madde ve kök kuru ağırlığı bakımından en yüksek değerlerin sırasıyla 33,64 cm, 2,29 ve 0,83 g bitki^{-1} ile stres uygulanmayan koşullarda KSC7158 genotipinde gözlendiği belirlenmiştir. Şiddetli kuraklık koşulları, klorofil içeriği ile göreli su içeriğinde azalmaya neden olmuştur. KSC7158 genotipi, en yüksek toplam klorofil ve göreli su içeriğine sahip olmuştur. Kuraklık şiddeti 8 bar'a ulaştığında, bitkilerde toplam çözünür şeker ve prolin konsantrasyonu artmıştır. En yüksek prolin içeriği KSC647 genotipinde, en yüksek toplam çözünür şeker ise KSC7158 genotipinde tespit edilmiştir. Ayrıca, 8 bar kuraklık stresinde katalaz, askorbat peroksidaz ve polifenol oksidaz enzim aktivitelerinde artış kaydedilmiş; KSC7158 ve

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KSC706 genotipleri bu enzimler açısından en yüksek aktivite düzeylerini sergilemiştir. KSC7158, KSC706 ve KSC201 hibritleri hem normal hem de şiddetli kuraklık koşullarında üstün büyüme performansı göstermiştir. Özellikle KSC7158 hibriti, incelenen tüm özellikler bakımından şiddetli kuraklık koşullarında en dayanıklı genotip olarak belirlenmiştir.

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INTRODUCTION

Maize (*Zea mays* L.), after the cereals rice and wheat, is the world's third most important cereal and forage crop and is designated as the "King of grain crops" (Yousaf et al., 2023). Maize is a valuable material that is planted for its high energy, fiber, crude protein, and nutritional value. Silage feeding has the potential to improve nutritional value and lower feeding expenses without compromising the animals' physiology or performance (Kökten et al., 2017; Kara and Sürmen, 2018; İleri et al., 2022). After wheat and rice, maize has the largest cultivation area in the world, with a production area of approximately 185 million ha (Molla et al. 2023). When total production is taken into account, it ranks first, ahead of wheat and rice, with a production of 1 billion tons (Çağlar et al., 2017). Eight to ten percent of the plant is consumed by people as byproducts, such as glucose, starch, and maize oil. In addition, it provides food, feed, and fodder (Shojaei et al., 2022). Silage maize is a valuable forage resource with high energy, fiber, protein concentration, and valuable minerals (İleri et al., 2022). Following fermentation, maize starch is also utilized globally as biofuel (as ethanol). Maize is one of the most important economic crops in the world, and about 129 million hectares of the world's fields, with an average yield of 2.4 tons per hectare (in developing countries) to 6.7 tons per hectare (in developed countries) are under the cultivation of this crop (Yousaf et al., 2023).

One of the main causes of decreased crop productivity and agricultural output is stressful circumstances. Addressing the impacts of stress is thought to be one of the effective ways to boost production (Shojaei et al., 2022). Stress from drought is one of the most significant environmental factors that lowers and restricts crop productivity among environmental stresses. Plants typically have an unstable supply of water throughout the course of their lives due to continuously changing environmental variables (Heydarzadeh et al., 2022). The reactions of plants to moisture stress are determined by the type of plant, years of age, maturity and growth phase, shortage intensity and duration, and physical characteristics (Yadav et al., 2023). Seasonal shortages are one of the most significant aspects restricting global maize cultivation and production (Tiwari et al., 2023). Drought lowers the world's annual yield of maize by 17% on average, and in some years, it has been reported to reduce production by over 70% (Yousaf et al., 2023). Yousaf et al. (2023) described differences between corn cultivars in terms of resistance to water deficit, which in resistant cultivars was not due to a better ability to absorb water from the soil, but rather the higher water use efficiency in these cultivars, which led to higher yields.

Maize production is significantly affected when the crop is cultivated under drought stress conditions. Water shortage stress is mainly common in areas experiencing negative effects of climate change, which causes environmental abiotic stresses due to increased temperatures (Shahzad et al., 2023). Water deficit stress (drought) decreases the water supply in the soil through evapotranspiration (Heydarzadeh et al., 2023a). It is difficult for investigators to improve tolerance to drought because, based on the degree of stress caused by water and how long the plant experiences it, the growth itself uses various coping mechanisms to deal with stress (Sandhu et al., 2023). Therefore, the best course of action in situations of water deficiencies is to increase the productivity of water in relation to crop production per unit area (Rahimi et al., 2022). It can negatively affect them at the morphological, physiological, biochemical, and molecular levels (Molla et al. 2023). We can learn more about the processes of adjusting to a harsher environment by studying biochemical characteristics under stressful circumstances (Mannan et al., 2023). Another major factor limiting productivity in agriculture is the shortage of drought stress. Crop growth is negatively impacted by a lack of drought stress, which is indicated by a decrease in the capacity for photosynthesis and an increase in the senescence of leaves (Kumdee et al., 2023). Under conditions of water deficit stress during photosynthesis, there is an increased leakage of electrons towards oxygen (O2), which results in the generation of various reactive oxygen species (ROS). These include superoxide, hydrogen peroxide, hydroxyl radicals, and oxygen radicals. (Heydarzadeh et al., 2024). Various morphological changes, physiological, and biochemical processes are developed by crops to prevent or eliminate the adverse impacts of stress. To deal with

ROS-induced oxidative damage, crops have both enzymatic and non-enzymatic antioxidant responses (Heydarzadeh et al., 2022). Other effects of stress due to water involve reduced growth of cells, expansion of leaves, adjustment of translocation and transpiration, and the production of proteins and chlorophyll (Mannan et al., 2023). In addition, stress caused by water reduces net absorption of carbon dioxide and most biological functions in maize crops (Kumdee et al., 2023).

In this regard, this study aims to evaluate the level of phenotypic and genotypic diversity of different traits among the investigated hybrids, especially in terms of drought resistance, to estimate the yield reduction due to drought stress, and to evaluate the direct and indirect effects of traits affecting yield under drought stress. The results of this study can be utilized in creating approaches to management for increasing the widespread production of maize with more effective hybrids under stressful circumstances.

MATERIAL and METHOD

Experimental Design

The study was conducted at the research greenhouse of Mohaghegh Ardabili University in Iran, located at coordinates 45°10' E and 37°44' N, with an elevation of 1338 meters above sea level. The experimental design employed a factorial arrangement based on a randomized complete block design in three repetitions, incorporating seven maize varieties (KSC201, KSC704, KSC705, KSC706, KSC7158, KSC647, and TWC647) and four levels of drought stress (0, 2, 4, and 8 bar), with three replications for each treatment.

Plant Material

The Ardabil Agricultural and Natural Resources Research and Education Center, AREEO, Moghan, provided the maize seed strains required for the present study. Grains were sterilized for one and a half minutes in 96% ethanol before being washed four times in pure water. This was carried out during the spring season (1 May to 10 September 2021). The maize seeds were subsequently planted in a plastic container (10 kg) with a diameter of 30 cm and a height of 25 cm and were randomly placed in the greenhouse, which contained a mix of soil and perlite (2:1, v:v) that had been watered prior to sowing. The pots were maintained in greenhouse conditions with a daily temperature range of 25±2°C and a night temperature of 15±3°C, with a light period of 15-16 hours and a dark period of 8 hours, and a humidity of 40-50%. Three weeks after sowing, seedlings of maize were trimmed to six in each pot. To determine the soil moisture level of the pots to apply irrigation treatments, the soil moisture level at different soil water potentials was determined using a pressure plate device, and then a pressure plate moisture curve was prepared. After that, the soil water potential of the pots was determined by weighing the soil of six pots randomly daily. During the study, equal amounts of irrigation were provided according to requirement. Based on the results of soil analysis, the soil had a nitrogen content of 0.04%, a pH of 8, potassium content measuring 250 mg kg^{-1}, and phosphorus content of 12.08 mg kg^{-1}. According to the findings of the soil test, 200 kg ha^{-1} of urea and 150 kg ha^{-1} of phosphorus fertilizer (triple superphosphate) were put into the soil and mixed. The environment's temperature for the period of study ranged between 17 and 20 degrees Celsius. Physiological and biochemical parameters were determined during the phase of flowering, and gathering was completed at the conclusion of the growth period.

Measurements

Growth Parameters

In the preliminary growth phase, several growth parameters were evaluated, including plant height (PH), total dry weight (TDW), and root dry weight (RDW). The plant samples were subjected to an oven-drying process at 70°C for a duration of 48 hours to ensure the complete removal of moisture. Following the drying procedure, the samples were weighed using a precision scale. To measure the fresh weight of roots and shoots, three plants were randomly selected from each replicate, and after separating the roots and shoots from each other, each was weighed to obtain the fresh weight of the shoots using a laboratory balance (Sarto S214TE rius model) with an accuracy of 0.0001 g. The longitudinal growth of the shoots was measured with a millimeter ruler. To measure the dry weight of the shoots, the fresh tissue was placed in an oven at 70 °C for 48 hours and then weighed using a laboratory balance (Ibrahim et al., 2022).

Relative water content (RWC)

The relative water content (RWC) of the leaf was assessed utilizing the methodology outlined by Holaday et al. (1992). (Eq. 1). Three leaves from each plant were randomly selected. Then, the same disks with a diameter of two cm were used to measure RWC.

$$\% RWC = [(FW–DW)/(TW–DW)] \times 100 \quad (1)$$

The leaves were immersed in purified water for a duration of 16 to 18 hours subsequent to the assessment of their fresh weight (FW). Following this, the turgid material was quickly blotted dry to remove any excess surface water, allowing for the calculation of the turgid weight (TW). Subsequently, the samples were subjected to oven drying at 70°C for a period of 24 hours in order to ascertain their dry weight (DW).

Total Chlorophyll (Total Chl)

Fresh leaf tissue (0.5 g) collected was pulverized in a nitrogen solution, mixed with 10 mL of 80% acetone, and homogenized by centrifugation at 4000 rpm for 15 min. Subsequently, Chlorophyll a and chlorophyll b concentrations were determined spectrophotometrically according to the following equations (Arnon, 1967).

$$\text{Chlorophyll a} = 11.24 \times A662 - 2.04 \times A645$$
$$\text{Chlorophyll b} = 20.13 \times A645 - 4.19 \times A662$$

Where, A622 and A645 are the absorbances at the wavelengths of 622 and 645 nm, respectively.

Electrical Conductivity (EL)

To get rid of surface-adhered electrolytes, newly collected (not frozen) leaves were rinsed three times with water that was deionized. At 25°C, 0.1 g of leaves were sliced into uniformly sized discs and incubated in a solution of deionized water. A conductivity analyzer was used to determine the absorption solution's electrical conductivity (L1) after one hour. After 10 minutes at 100 °C in a hot water bath, the water-soluble solution was cooled, and the electrical conductivity (L2) was evaluated. The equation L1/L2 × 100% was used to determine the relative permeability of the cell membrane (Dionisio-Sese and Tobita, 1998).

Proline Content

Leaf proline content was determined using the ninhydrin colorimetric method. Leaf tissue (0.5 g) was finely powdered in a mortar using liquid nitrogen, homogenized in 10 mL of 3% sulfosalicylic acid solution, and centrifuged at 4.000 rpm for 15 minutes to obtain a clear supernatant. Then, a glacial acetic acid solution of proline ninhydrin acid was prepared (1:1:1, v:v:v) and equilibrated at 100°C for one hour to facilitate the reaction between proline and ninhydrin, forming a chromophore. The reaction was stopped by rapidly cooling the solution in an ice bath. To develop the chromophore, 4 mL of toluene was added to the reaction mixture, enabling the extraction of the chromophore into the organic phase. Finally, the absorbance of the samples was measured at 515 nm using a spectrophotometer. L-proline was used as a standard to make a reference curve (Bates 1973).

Total soluble sugars (TSS)

The total soluble sugar (TSS) content in leaves was evaluated through the phenol-sulfuric acid method. Leaf tissue (0.5 g) was powdered in a mortar using nitrogen solution, mixed with ethanol, and combined with 5% phenol. Next, 5 mL of 98% sulfuric acid was added to the mixture and incubated for one hour before measuring the absorption of the solution at 625 nm using a spectrophotometer. A standard curve was generated by plotting solutions with varying D-glucose concentrations (Irigoyen et al., 1992).

Enzyme Extractions and Assays

To measure antioxidant enzyme activity, 100 mg of fresh material was finely powdered and treated with 2 mL of 0.1 M KH_2PO_4 buffer containing 5% polyvinylpyrrolidone (PVP) at pH 6. The extracts were centrifuged at 15.000 rpm for 30 minutes at 3°C, and the clear supernatant was used to determine before the protein content by the Bradford method, and then, the specific activity of the enzyme as reported below.

Catalase Activity (CAT)

The approach of Chance and Maehly (1959) was used to measure the pace at which hydrogen peroxide vanished in order to assess CAT activity. Catalase (CAT) activity was measured at 240 nm through the variation in hydrogen peroxide (H_2O_2) concentration. The reaction mixture contained 1.9 mL of 50 mM K_3PO_4, which was buffered at a pH of 7, 10 mM H_2O_2, and 0.2 mL of enzyme extract. Enzymatic activity was then measured in 60 seconds per mg of protein based on absorption variations.

Polyphenol oxidase (PPO) activity

With some adjustments, the Kar and Mishra (1976) protocol was used to quantify the activity of polyphenol oxidase

(PPO). The 3 ml reaction mixture contained 25 mM phosphate buffer (pH 6.8), 0.1 mM pyrogallol, 0.1 ml enzyme extract, and a blank without pyrogallol. The absorbance of the purpurogallin formed was recorded at 420 nm.

Ascorbate peroxidase activity (APX)

Ascorbate peroxidase activity (APX) was assessed utilizing the protocol established by Kar and Mishra (1976). The ascorbate peroxidase (APX) activity was measured using a reaction mixture containing 1 mL of 0.5 mM ascorbic acid, 1 mL of 100 mM K_3PO4 buffered at a pH of 7, 100 µL of enzyme extract, and 0.1 mL H_2O_2 0.1 mM. The absorption was then read at 240 nm.

Data Analysis

The assumptions of normality and homogeneity of variances were checked before performing ANOVA. SAS 9.1 software was used to statistically evaluate the data produced in this investigation. Graphs were created in Microsoft Excel, and means were compared using Tukey's HSD at the $p < 0.05$ level.

RESULT and DISCUSSION

The interaction between genotype and drought stress significantly influenced the plant height (PH), total dry weight (TDW), and root dry weight (RDW) of maize at a probability level of $p < 0.01$ (Table 1). Furthermore, the analysis of Total Chlorophyll (Total Chl), Relative Water Content (RWC), proline, Total Soluble Solids (TSS), Electrolyte Leakage (EL), Catalase (CAT), and Ascorbate Peroxidase (APX) was affected by the individual effects of both genotype and drought stress, also at a probability level of $p <0.01$ (Table 1). In contrast, EL and Polyphenol Oxidase (PPO) were significantly influenced solely by the individual effects of genotypes at the same probability level of $p < 0.01$ (Table 1).

Table 1. Variance analysis of morphological and physiological traits of maize genotypes under drought stress.
Çizelge 1. Kuraklık stresi altında mısır genotiplerinin morfolojik ve fizyolojik özelliklerine ait varyans analizi.

Source of variation	df	Mean squares										
		PH	TDW	RDW	Total Chl	RWC	Proline	TSS	EL	CAT	APX	PPO
Genotype (G)	6	293**	126**	12**	0.07**	941**	5.46**	139**	33**	0.03**	0.03**	0.03**
Drought stress (Ds)	3	284**	63**	4.50**	0.02**	618**	1.98**	84**	6.31^{ns}	0.021*	0.06**	0.011^{ns}
G×Ds	18	7.89**	1.27**	0.18**	0.004^{ns}	1.39^{ns}	0.0002^{ns}	0.29^{ns}	1.66^{ns}	0.01^{ns}	0.01^{ns}	0.006^{ns}
Error	56	0.27	0.22	0.08	0.004	9.18	0.003	3.20	2.34	0.006	0.006	0.005
CV (%)		2.15	2.92	4.36	7.30	4.67	2	9.44	10.49	20.84	23.30	6.50

**, * and ns, significant at 1% and 5% levels of probability, non-significant, respectively.
PH: plant height; TDW: total dry weight; RDW: root dry weight; Total Chl: Total chlorophyll; RWC: Relative water content; TSS: Total soluble sugars; EL: Electrolyte leakage; CAT: catalase activity; APX: ascorbate peroxidase activity; PPO: Polyphenol oxidase.

Growth parameters

The highest plant height (PH), total dry weight (TDW), and root dry weight (RDW) (33.64, 2.29, and 0.83 g plant^{-1}) were recorded in genotype KSC7158 under no-drought stress conditions, respectively (Figure 1). The lowest values were 14.73, 0.98, and 0.49 g plant^{-1}, observed from cultivar KSC647 under 8-bar conditions (Figure 1). However, PH, TDW, and RDW in KSC7158 and KSC706 cultivars were significantly higher than in other cultivars in 8-bar conditions (Figure 1). Stress caused by a lack of moisture has a negative impact on plants because it lowers cell viability and prevents cell division, elongation, and expansion, which lowers vegetative and morphological growth. The crop's potential for water may rise as a result of the suppression of Rubisco activity, which increases the ability to resist carbon dioxide release and lowers stomatal capacitance under moisture scarcity stress (Ibrahim et al., 2022). The preferred distribution of material to the roots as a result of moisture constraints or a decreased chlorophyll content, which lowers photosynthetic efficiency, could be the cause of the observed decline in dry weight yield with rising drought stress (Heydarzadeh et al., 2023a). Plants that are under stress from a lack of moisture try to reduce evaporation by closing their stomata, which results in fewer gas exchanges (Yavuz et al., 2022), lower CO_2 intake, and higher oxygen levels. Ultimately, this lowers Rubisco's carboxylation activity, which in turn reduces the synthesis of dry matter (Sandhu et al., 2023). KSC7158, KSC201, and KSC706 maize varieties had greater plant height, total dry weight, and root dry weight than KSC647, KSC704, and KSC705 cultivars (Figure 1). This beneficial outcome is explained by increased leaf surface space and enhanced leaf resilience, which increase accessibility of nutrients and uptake and boost crop development, growth, and production in conditions of moisture scarcity (Shahzad et al., 2023).

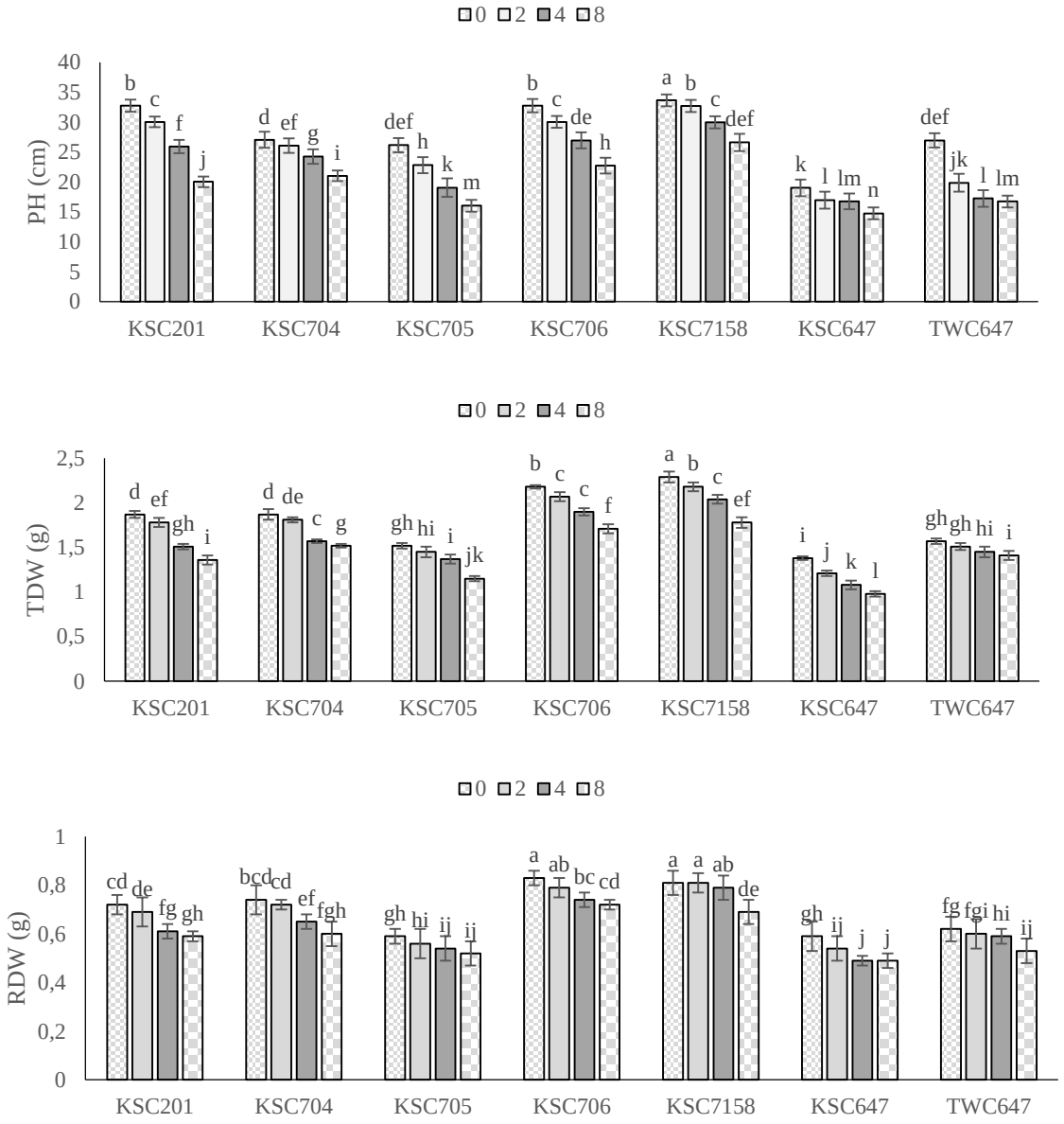


Figure 1. The effects of drought stress and maize varieties on plant height, total dry weight, and root dry weight. The same letters show a non-significant difference at P ≤ 0.05 by Tukey's HSD test.

Şekil 1. Kuraklık stresi ve mısır çeşitlerinin bitki boyu, toplam kuru ağırlık ve kök kuru ağırlığı üzerindeki etkileri. Aynı harfleri taşıyan değerler, Tukey HSD testiyle P ≤ 0.05 düzeyinde anlamlı fark göstermemektedir.

Chlorophyll (Total Chl) content

The drought stress decreased the amount of total chlorophyll (Total Chl), so that the highest Total Chl (0.90 mg/g FW) was noted in the absence of drought stress. Also, the lowest Total Chl (0.74 mg/g FW) was obtained in 8-bar conditions (Figure 2). Water scarcity and the generation of reactive oxygen species, which break down and peroxidize chlorophylls, are the causes of the decrease in chlorophyll content during stressors involving moisture limitation (de Oliveira Neto et al., 2023). According to Kumdee et al. (2023), ROS are known to break down proteins and lipids, limit the number of photosynthetic pigments, and weaken the chloroplast membranes, which causes them to break down. However, under stress from drought, the concentrations of chlorophyll a and chlorophyll b increased in the KSC7158, KSC201, and KSC706 maize types (Figure 2). The increased intake of nutrients, particularly nitrogen, by the KSC7158, KSC201, and KSC706 maize varieties provides the nitrogen needed to produce photosynthetic pigments and plant cell protein molecules, which increases the concentration of these pigments in crops since chlorophyll and carotenoids are perpetually bound to proteins (Darwish et al., 2023; Yadav et al., 2023; Kumdee et al., 2023). Crop yields improve as a result of this increase.

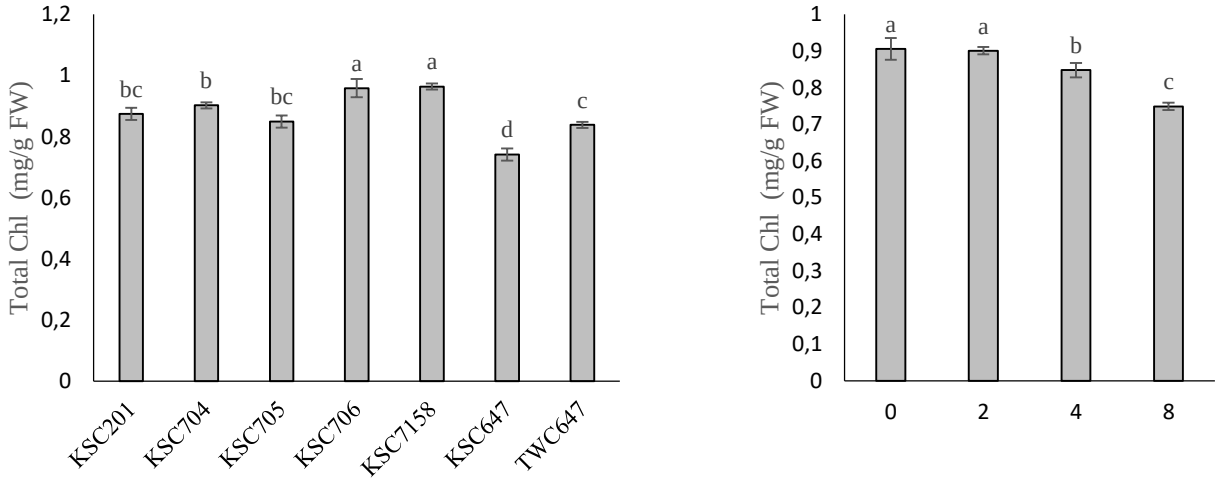


Figure 2. The effect of drought stress and maize varieties on the content of total chlorophyll.
The same letters show a non-significant difference at P ≤ 0.05 by Tukey's HSD test.

Şekil 2. Kuraklık stresi ve mısır çeşitlerinin toplam klorofil içeriği üzerindeki etkisi.
Aynı harfleri taşıyan değerler, Tukey HSD testiyle P ≤ 0.05 düzeyinde anlamlı fark göstermemektedir.

Relative water content (RWC)

The highest relative water content (RWC) (75.15%) was obtained from the KSC7158 cultivar. The lowest one (53.06%) was obtained from the KSC647 cultivar. On the other hand, KSC705 and TWC647 cultivars showed comparable values of RWC (Figure 3). Similarly, there was no difference in RWC between KSC704 and KSC201 cultivars (Figure 3). The RWC in control plants was greater than in 8-bar conditions (Figure 3). The RWC of leaves under stress conditions and drought stress of 8 bar conditions was 71.49 and 56.13%, respectively (Figure 3). The decrease in RWC has been attributed to a number of significant variables, including decreased growth and root activity as well as enhanced transpiration and evaporation from the area covered by crops (Ali et al., 2023). Decreased consumption of water by the roots and raised transpiration via the surface of the leaves, which eventually causes the closure of leaf stomata, are the main causes of a reduction in leaf RWC brought on by harsh irrigation regimens (Kumdee et al., 2023). Plant tissue turgor and RWC declines might be early indicators of stress that directly affect cell growth and division (Gopalakrishna et al., 2023). But in every irrigation regime, the RWC was greatly enhanced by the KSC647, KSC704, and KSC705 cultivars, which also had favorable effects on root growth, evaporation/transpiration ratio, leaf water capacity, and absorption of water (Ljubičić et al., 2023).

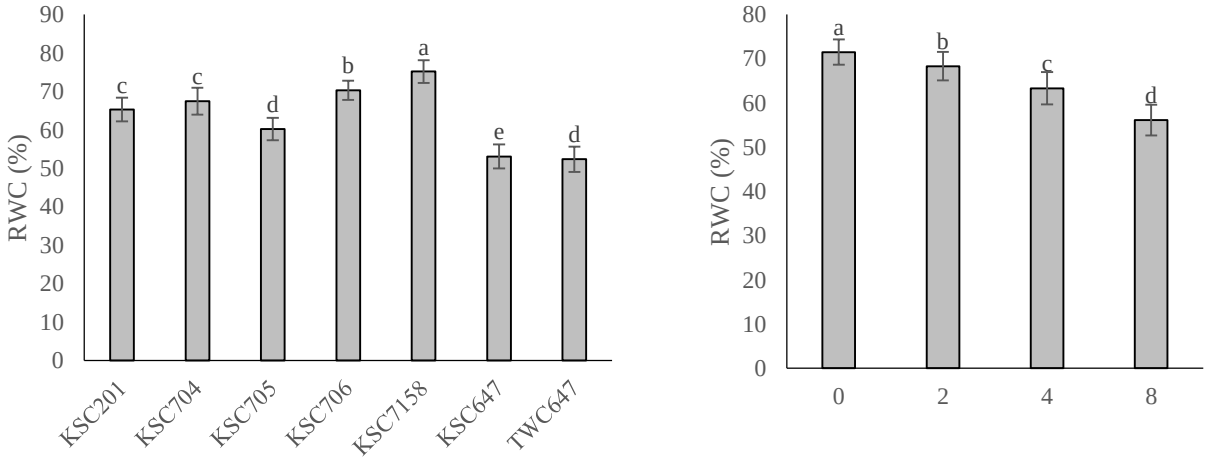


Figure 3. The effect of drought stress and maize varieties on relative water content.
The same letters show a non-significant difference at p ≤ 0.05 by Tukey's HSD test.

Şekil 3. Kuraklık stresi ve mısır çeşitlerinin göreli su içeriği üzerindeki etkisi.
Aynı harfleri taşıyan değerler, Tukey HSD testiyle P ≤ 0.05 düzeyinde anlamlı fark göstermemektedir.

Proline content

The highest proline (3.52 µmol/g fw) was observed under drought stress at 8-bar conditions. The lowest was 2.41 µmol g^{-1} FW acquired in control plants (Figure 4). The highest proline (3.48 µmol g^{-1} FW) was acquired from the KSC647 cultivar. The lowest one (2.39 µmol g^{-1} FW) was obtained from the KSC7158 cultivar (Figure 4). The most prevalent and well-known accumulation of compounds that help maintain cellular water balance, proline, is known to accumulate when subjected to stress caused by a lack of moisture, offering protective effects on cytosol enzymes and cell structure (Wu et al., 2023). The regulation of ABA on the light-dependent absorption involved in proline synthesis has been linked in a few studies to this reaction (Kumdee et al., 2023), while other investigations have linked it to higher-energy photosynthetic organism materials that facilitate the mechanism of proline synthesis (Heydarzadeh et al., 2023b). According to Hussain et al. (2023), accumulation of proline is thought to be the primary osmotic balance pathway that improves crop stress resistance. Under conditions of moisture scarcity, KSC647, KSC704, and KSC705 cultivars typically show more effective nutrient and water management. Consequently, KSC647, KSC704, and KSC705 cultivars reduce proline content by raising the number of root branches, affecting plant cell metabolism, improving chelating capacities, and improving water and nutrient absorption (Yousaf et al., 2023; Owusu et al., 2023).

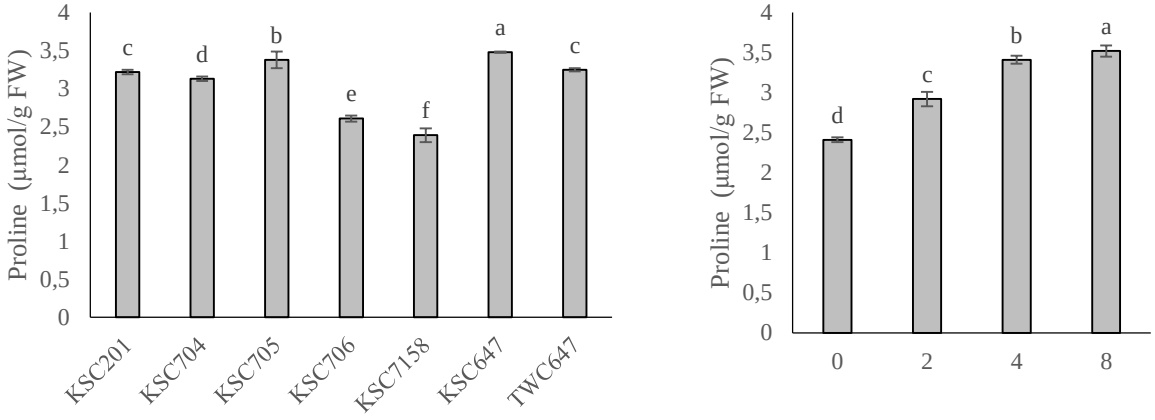


Figure 4. The effect of drought stress and maize varieties on proline.
The same letters show a non-significant difference at $p \leq 0.05$ by Tukey's HSD test.
Şekil 4. Kuraklık stresi ve mısır çeşitlerinin prolin içeriği üzerindeki etkisi.
Aynı harfleri taşıyan değerler, Tukey HSD testiyle $P \leq 0.05$ düzeyinde anlamlı fark göstermemektedir.

Total soluble sugars (TSS)

The study revealed that the concentration of total soluble sugars (TSS) in 8-bar conditions was significantly higher than that observed in non-stressed conditions (Figure 5). The KC7158 cultivar exhibited the highest concentration of TSS, measuring 21.68 mg/g FW, while the KSC 647 cultivar was recorded the lowest concentration at 13.6 mg/gFW (Figure 5). As a possible tactic for the growth of plants in scarce water situations, watering delay increased the amount of TSS in oregano leaves. The hydrolysis of carbon stores is the main cause of TSS (Gopalakrishna et al., 2023). The formation of TSS is impacted by the balance between photosynthesis and growth, as both processes are impacted by stress caused by drought (Wu et al., 2023). The buildup of products from photosynthesis can be anticipated since growth is impacted before photosynthesis is hampered by water scarcity (Kumdee et al., 2023). Accordingly, at tiny amounts, sucrose functions as a signaling molecule, while at high quantities, it scavenges reactive oxygen species (Hussain et al., 2023). In their capacity as osmotic regulators, the soluble sugars control osmosis and lower the water capacity. As a result, they increase a plant's ability to withstand situations of water deficit by helping it store more water in its cells and preserve turgor under stress from drought (Mannan et al., 2023). KSC647, KSC704, and KSC705 cultivars showed higher quantities of soluble sugars, which boosted vegetative development and raised the amount of carbohydrates (Mannan et al., 2023). Additionally, by preserving the equilibrium of carbon and increasing osmotic adjustment, the greater sugar amount in this instance probably helped to improve resistance to drought and plant responses to water stress (Ljubičić et al., 2023).

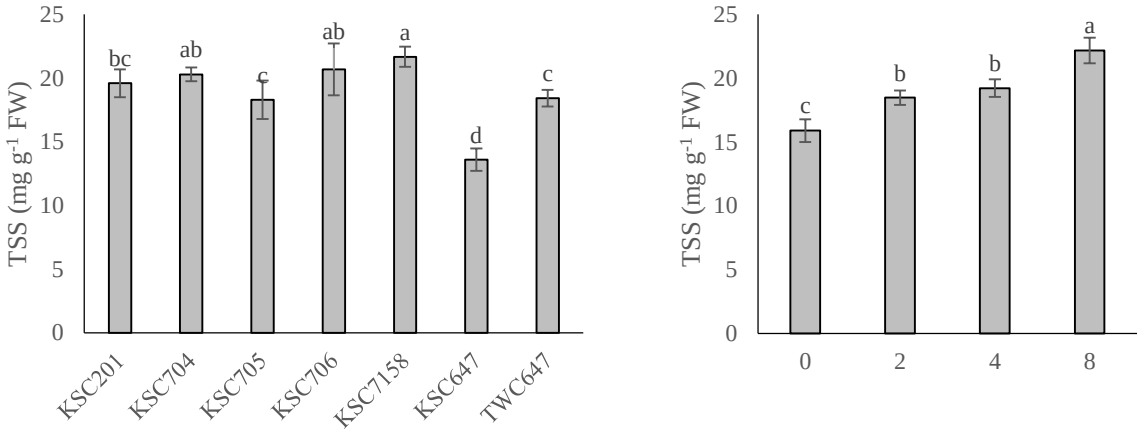


Figure 5. The effect of drought stress and maize varieties on total soluble sugar.
The same letters show a non-significant difference at $p \leq 0.05$ by Tukey's HSD test.
Şekil 5. Kuraklık stresi ve mısır çeşitlerinin toplam çözünür şeker içeriği üzerindeki etkisi.
Aynı harfleri taşıyan değerler, Tukey HSD testiyle $P \leq 0.05$ düzeyinde anlamlı fark göstermemektedir.

Electrolyte leakage (EL)

The study revealed that the highest electrolyte leakage (EL) was 17.21 ds/m^3 (Figure 6), which was observed in the KSC647 genotype. The lowest EL (12.79 ds/m^3) was observed in cultivar KSC7158, which did not differ significantly from cultivar KSC706 (Figure 6). However, EL in KSC647 and KSC705 cultivars was significantly higher than in other cultivars (Figure 6). As a result, EL is calculated to estimate how stress from drought affects membrane permeability (Darwish et al., 2023). The cultivars KSC647 and KSC705 that we examined in our studies both displayed increased EL, although KSC647's EL was noticeably higher. In maize, severe drought significantly raised oxidative markers and EL, while more resistant cultivars displayed a decline in these metrics (Tiwari et al., 2023).

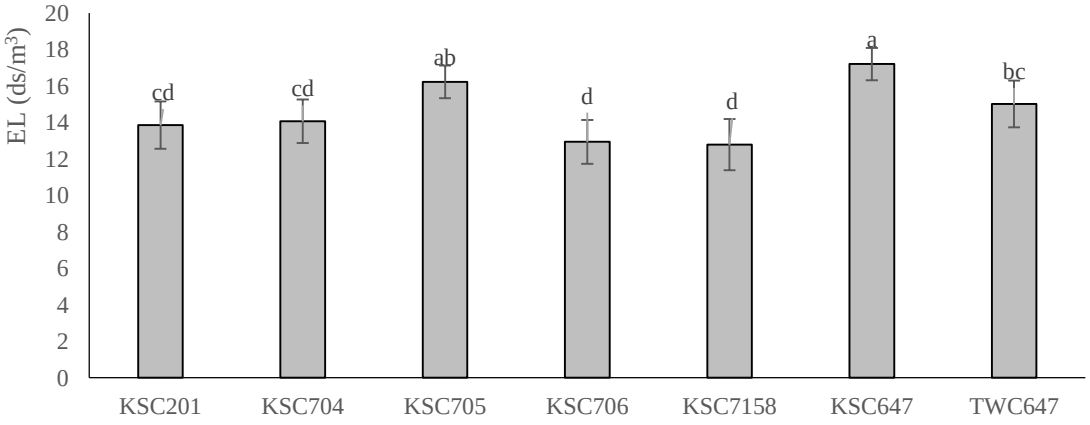
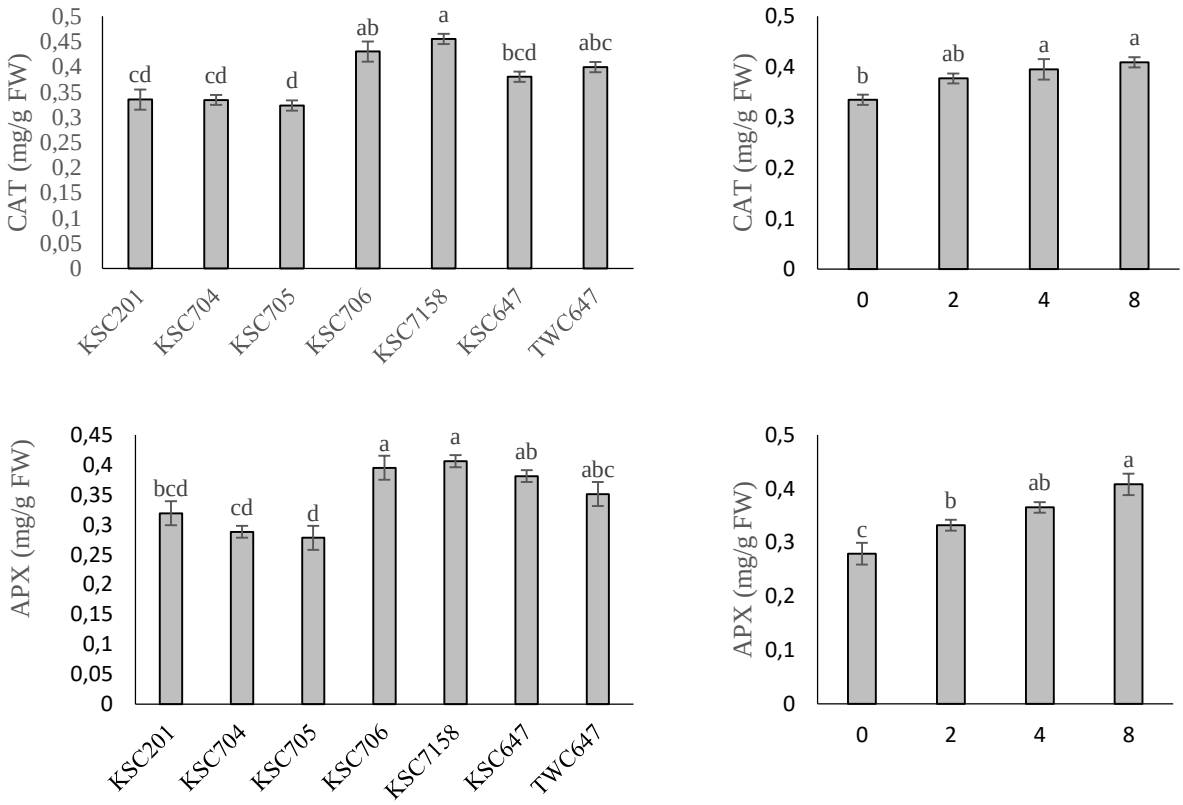


Figure 6. The electrolyte leakage of maize varieties.
The same letters show a non-significant difference at $P \leq 0.05$ by Tukey's HSD test.
Şekil 6. Mısır çeşitlerinin EL ortalamalarının karşılaştırılması.
Aynı harfler Tukey's HSD testi ile $P \leq 0.05$'te önemli olan farkı göstermektedir.

Antioxidant Enzymes Activity

The highest activity of catalase (CAT), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) activity (0.45, 0.40, and 0.28 mg g^{-1} FW) was in genotype KSC7158. The lowest was 0.32, 0.27, and 0.15 mg g^{-1} FW observed from cultivar KSC705 (Figure 7). The drought stress raised the activity of CAT, and APX, notably different from the control (Figure 7). The highest activity of CAT, and APX (0.409 and 0.408 mg g^{-1} FW) was noted in drought stress of 8 bar. Additionally, under severe drought circumstances, the 8-bar therapy had no discernible impact on CAT activity in comparison to the 4-bar (Figure 7). However, the lowest activity of CAT and APX (0.335 and 0.279 mg g^{-1} FW) was obtained from control plants (Figure 7). In accordance with Heydarzadeh et al. (2023a), who found

that water shortages in dragon's head plants caused the production of antioxidant enzymes, the activity of CAT, SOD, and PPO enzymes was elevated in maize plants with growing water scarcity. The ability of plants to activate their protective antioxidant systems is, in fact, strongly correlated with stress caused by a lack of moisture (Hussain et al., 2023). A plant's mechanism for defense against a variety of biotic and abiotic challenges is the enhancement of antioxidant enzyme activity. When plant cells are stressed by a lack of moisture, ROS can build up and harm lipids, nucleic acids, carbohydrates, and membranes, and cause metabolic changes (Yousaf et al., 2023). Plant cells use an enzymatic defensive approach to detoxify and remove ROS, increasing their resistance to moisture deficiency stress (Gao et al., 2023). More precisely, in order to eliminate excess ROS and stimulate their defense system, crops produce and attract the antioxidant enzymes CAT and SOD (Heydarzadeh et al., 2022). By turning O_2^- into H_2O_2 and O_2, SOD and PPO are the first line of defense against ROS degradation when there is a water deficiency (Tiwari et al., 2023; Gong et al., 2023). High quantities of H_2O_2 can harm cells, despite the fact that low amounts are advantageous since they function as an intracellular signal (Rahimi et al., 2022). Therefore, the turning of H_2O_2 into H_2O and other non-toxic compounds is catalyzed by the CAT enzyme at this point (Rahimi et al., 2022). CATs are crucial enzymes for antioxidant defense that, in stressful conditions, detoxify cells from ROS and convert "H_2O_2" into H_2O (Heydarzadeh et al., 2023a). According to earlier research, maize cultivars' CAT, SOD, and APX activities rose when soil moisture content decreased, demonstrating the plants' growing resistance to water deficiency (Molla et al., 2023). According to these findings, cultivars of KSC647, KSC704, and KSC705 may be able to reduce oxidative stress by preserving stable levels of ROS between cells and so shielding cell membranes from harm brought on by extreme water deprivation (Kisman et al., 2023; Kumdee et al., 2023). By altering the expression of particular plant genes involved in defense mechanisms against stressful situations, KSC647, KSC704, and KSC705 cultivars can also affect tolerance to water deficiency and unfavorable external conditions (Emam et al., 2023; Shahzad et al., 2023).



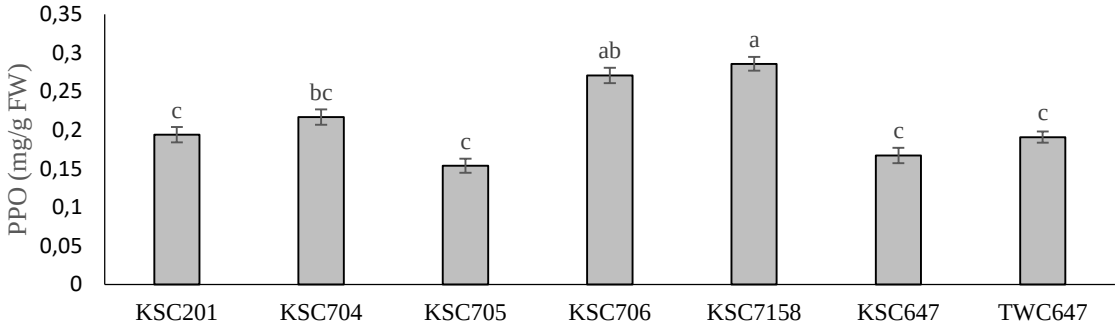


Figure 7. The effect of drought stress and maize varieties on CAT, APX, and PPO.
The same letters show a non-significant difference at $P \leq 0.05$ by Tukey's HSD test.

Şekil 7. Kuraklık stresi ve mısır çeşitlerinin CAT, APX ve PPO enzim aktiviteleri üzerindeki etkisi.
Aynı harfleri taşıyan değerler, Tukey HSD testiyle $P \leq 0.05$ düzeyinde anlamlı fark göstermemektedir.

CONCLUSION

Maize genotypes were negatively impacted by drought stress, which decreased plant height, total dry weight, root dry weight, and leaf surface. However, it increased the concentration of total soluble sugars, proline, and the antioxidant activity of enzymes. KSC7158, KSC706, and KSC201 were identified as drought-tolerant genotypes, and KSC704, KSC705, KSC647, and TWC647 genotypes were identified as susceptible genotypes based on the physiological characteristics of the study in this experiment. Finally, it can be concluded that the KSC7158, KSC706, and KSC201 were superior to other hybrids in terms of growth parameters under normal conditions and stress, and the KSC7158 was identified as the most drought-tolerant genotype. This study was conducted under greenhouse conditions and should be supported by open-field experiments.

Author Contributions

Execution research project, Experimental design, Data analysis. NA, SJ, SF, SH; Manuscript preparation, writing, review & editing. NA, SJ, SF, EK, SH, MS

Conflict of Interest

The authors declare that they do not have any competition and any conflicts of interest.

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