RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
56 (6) 2341-23500, 2024
http://doi.org/10.54910/sabrao2024.56.6.15
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

DROUGHT TOLERANCE ASSESSMENT IN MAIZE HYBRIDS: MORPHOPHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION

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SUMMARY

Climate change caused drought stress to become a critical challenge in maize (*Zea mays* L.) production, surpassing other environmental stresses. Understanding drought resilience mechanisms in maize is essential for breeding drought-tolerant varieties. This study evaluated the drought tolerance of two maize hybrids, Sancia and Agrister, under greenhouse conditions with four irrigation regimes: 90%, 75%, 50%, and 25% of field capacity (FC). Under severe drought (25% FC), the hybrid Agrister showed reductions in shoot length, leaf area, biomass, and chlorophyll content by 13.9%, 29.86%, 30.76%, and 13.7%, respectively, compared with well-watered conditions (90% FC). In both hybrids, electrolytic conductivity increased under drought, indicating membrane stress. Agrister, however, maintained lower levels of hydrogen peroxide (H_2O_2) than Sancia, signaling a better antioxidative balance. A higher catalase and peroxidase enzyme activity marked Agrister's response to drought, alongside increased phenolic compounds, strengthening its antioxidant defense system. The study concludes that Agrister's superior drought tolerance makes it a promising candidate for cultivation in Tunisia's arid regions, highlighting its potential in breeding programs focused on resilience to water scarcity.

Keywords: Maize (*Z. mays* L.), drought stress, chlorophyll content, biochemical traits

Key findings: Maize (*Z. mays* L.) hybrid Agrister excelled the hybrid Sancia under water-deficit conditions and showed better development of aerial parts and a higher drought tolerance. The genetic variability between the two maize hybrids highlights the potential for breeding drought-resistant maize.

Communicating Editor: Dr. Himmah Rustiami

Manuscript received: August 07, 2024; Accepted: November 23, 2024.

Citation: Kthiri Z, Hammami MDE, Jabeur MB, Marzougui O, Hamada W, Karmous C (2024). Drought tolerance assessment in maize hybrids: Morphophysiological and biochemical characterization. *SABRAO J. Breed. Genet.* 56(6): 2341-2350. http://doi.org/10.54910/sabrao2024.56.6.15.

INTRODUCTION

Maize (*Zea mays* L.) is a major cereal crop consumed globally as a staple and a primary fodder for livestock (Dei, 2017). Worldwide, maize cultivation occurs approximately across 197 million hectares, with significant areas in sub-Saharan Africa (SSA), Asia, and Latin America (Erenstein *et al*., 2022). In Tunisia, however, maize production remains limited, largely due to various abiotic stresses, making accurate data collection challenging. Grown primarily as livestock fodder, the average cultivated area for maize in Tunisia is about 1,453 hectares. In 2021, this area sharply declined to 1,150 hectares due to severe drought conditions (Epule *et al*., 2022).

Maize growth and yield incur significant effects from various abiotic stresses, including salinity, drought, and extreme high temperatures. The Mediterranean region, including Tunisia, experiences climate change impacts, marked by low rainfall, rising temperatures, and limited, poor-quality water resources (Aloui *et al*., 2022). Drought stress disrupts several metabolic and physiological processes essential to maize growth (Chávez-Arias *et al*., 2021). As a C4 plant, maize requires a steady water supply, averaging around 3,000 m^3/ha throughout its growth cycle; without this, it risks substantial yield losses (Cucci *et al*., 2019).

The maize susceptibility to water deficit conditions depends on the intensity and duration of drought stress and the crop stage, resulting in yield losses varying from 30% to 90% (Ahmad *et al*., 2020). Water stress during the vegetative stage can considerably impede maize plant growth and leaf area, leading to reduced yield (Song *et al.,* 2020). However, some maize genotypes exhibited tolerance to mild water stress due to low water requirements during the early vegetative growth and late grain-filling phases (Comas *et al*., 2019). The flowering phase emerged as the most vulnerable stage to water stress, resulting in decreased biomass and grain yield (Ahmad *et al*., 2020).

Plants possess a complex antioxidative-defense system to counteract oxidative stress caused by reactive oxygen species (ROS) generated during abiotic stresses, such as drought. This system includes enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX), which are crucial in ROS detoxification. SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide (H_2O_2), subsequently broken down into water and oxygen by CAT. POX contributes to H_2O_2 detoxification and biosynthesizes lignin, thereby strengthening cell walls (Zandi and Schnug, 2022).

In addition to enzymatic antioxidants, plants employ non-enzymatic molecules, such as, phenolic compounds, flavonoids, ascorbic acid, and glutathione. These compounds neutralize ROS and regulate signaling pathways that enhance stress tolerance. For instance, phenolic compounds act as photo protectors by absorbing excess light and reducing chlorophyll degradation under water-deficit conditions (Le-Gall *et al*., 2015). They also facilitate leaf rolling, an adaptive mechanism to conserve water.

The balance and efficiency of these antioxidative systems vary among plant species and genotypes, significantly influencing stress tolerance. For example, the maize hybrid Agrister demonstrated higher catalase and peroxidase activities, along with stable levels of phenolic compounds, under severe drought conditions than Sancia. This enhanced antioxidative response is critical for maintaining cellular integrity and metabolic functions, highlighting its superior resilience to oxidative stress (Parveen *et al*., 2019; Ali *et al*., 2024).

Numerous studies confirmed water deficit induced a significant increase in activities of SOD and CAT isozymes in maize genotypes (Huang *et al*., 2019). Although, increased SOD and CAT activities may efficiently scavenge the harmful ROS, leading to decreases in O_2, H_2O_2, and MDA contents in maize plants. Based on the above discussion, the presented study evaluated the effect of water deficit conditions on two maize hybrids and identified the morphophysiological and biochemical determinants conferring water stress tolerance at the maize juvenile growth stage.

MATERIALS AND METHODS

Plant material and procedure

Two maize (*Z. mays* L.) hybrid cultivars, Agrister and Sancia, served as the experimental material. The hybrid seeds procurement occurred with the Lima Grain Agricultural Seed Corporation in Verneuil, France, listed in the Tunisian seed catalog. The maize hybrid seeds' disinfection used 10% HCl for 10 min and then rinsed three times with distilled water. Subsequently, sowing the seeds continued in 2 L pots, with four seedlings per pot. The potting mixture comprises a blend of peat and perlite in a 2:1 (v/v) ratio. After 15 days of sowing (dps) had only one plant per pot maintained. The trial commenced in a semi-controlled environment where the temperature ranged from 30 °C to 35 °C, ensuring consistent and suitable growing conditions for the maize plants.

Stress application

The uniformed irrigation of plants transpired every two days with a 50% Hoagland's nutrient solution. At the experiment, the maximum soil water content measuring for each pot determined the difference between the pot's weight after draining excess water and its dry weight. Throughout the experiment, watering the pots depended on direct measurements of their weight, with adjustments made to align with the established water conditions for each regime. Half of the pots remained under well-watered conditions (100% pot capacity, PC), while the other half experienced induced water and nutrient stress beginning 25 days after sowing.

This stress implementation continued by progressively reducing the Hoagland's nutrient solution by 10% of PC every two days. After 10 days of stress (dps), applying the four drought-stress irrigation regimes (90%, 50%, 75%, and 25% of FC) followed. The stressed plants remained with this irrigation regime until the experiment concluded (long-term severe stress). The measurement of average decreases in both water content and nutrient supply proceeded throughout the study. The experimental layout was in a completely random design (CRD) with 20 replications. The distribution of prepared pots' had a rate of 20 pots/genotype for each treatment, and four pots/irrigation regime.

Morphophysiological measurements

The physiological traits measurement occurred after three weeks of stress application (21 dps) in the fully expanded leaves. The measurements were relative to shoot length (cm) and dry matter content (%) of the aerial part. The fresh weight (FW) of the aerial parts reached recording and then drying at 70 °C for 48 h to measure the dry weight rate percent (DW %). Determining the dry matter content followed the equation below:

$$\text{Matter content (\%)} = (\text{dry weight [mg]}/\ \text{fresh weight [mg]}) \times 100$$

The leaf area measurement used the Image J software (Version 1.46r) (Wayne Rasband, NHI, USA). Leaf chlorophyll content determination engaged a chlorophyll meter (Minolta SPAD 502, Japan) by recording an average of three values (SPAD units) taken from three different levels (top, middle, and bottom of the plant).

In leaves, measuring the relative water content (RWC) was according to Barr and Weatherly (1962), as follows:

$$\text{RWC (\%)} = ([\text{wet weight - dry weight}]/[\text{turgid weight \{g\}- dry weight}]) \times 100$$

Where:
FW = wet weight (g), DW = dry weight (g), and TW = turgid weight (g).

The electrolytic conductivity (EC%) detection followed the method according to Dkhil and Denden (2012). Washing the leaves with distilled water removed any residue or electrolyte that might adhere to the surface, with the leaves cut into small discs of uniform size. The samples' immersion in 10 ml of distilled water ensued, and then, incubated at room temperature. After 24 h, reading the first conductivity (C1) used a conductivity meter

(Fisher Scientific), with the leaf samples autoclaved after at 120 °C for 20 min. The second reading (C2) began after cooling the solution to ambient temperature. The electrolytic conductivity's calculation continued according to the following formula:

$$EL\ (\%) = (C1/C2) \times 100$$

Biochemical analysis

The H_2O_2 content extraction and measurement employed the method described by Hu *et al*. (2022). Fresh shoot tissues (0.1 g) from each sample received homogenization with 2 mL of 0.1% (w/v) cold trichloroacetic acid (TCA), centrifuged at 14,000× g for 15 min, and with the supernatant collected after. The absorbance of the reaction mixture containing 0.5 mL of the supernatant, 0.5 mL of 10 mM phosphate buffer (pH 7.0), and 1 mL of 1 M KI incur reading at 390 nm. Determining the H_2O_2 content used an extinction coefficient of 0.28 $\mu M^{-1} cm^{-1}$ and expressed as $\mu M\ g^{-1}$ FW.

The measurement of catalase (CAT) activity followed the methodology of Hu *et al*. (2022). In detail, 100 mg of leaf sample mixed with 5 mL of sodium phosphate buffer (100 mM, pH 7.0) bore thorough grinding. The homogenate's centrifugation followed at 15,000× g for 20 min. CAT activity detection included adding 0.2 mL of the crude enzyme solution to 3 mL of sodium phosphate buffer containing 0.2 mL H_2O_2 as a substrate. Measuring the decomposition of H_2O_2 was by the decline in absorbance at 240 nm. One unit, defined as the change in 0.001 absorbance units per minute, had the specific activity expressed as units per gram of FW.

The quantification of soluble phenolic compounds applied the Folin-Ciocalteu method. Foliar tissues (200 mg from the third leaf) underwent homogenization in an ice bath with 1.5 ml methanol. The homogenate received centrifugation at 10,000× g for 5 min. Adding the 100 µL of the supernatant continued to the reaction mixture containing 50 µL of sodium carbonate (20%), 1750 µL of sterile distilled water, and 250 µL of Folin-Ciocalteu reagent (Sigma-Aldrich, Germany). The mixture's incubation was at 40 °C for 30 min, reading the blue color at 760 nm using catechin as a standard (Sigma Aldrich, USA). The content of soluble phenolic compounds' expression was in mg-equivalents of catechin per gram of FW.

Using 500 mg of FW homogenized in 5 mL of 50 mM K-phosphate buffer (pH 5.5) determined the peroxidase activity. After centrifugation at 12,000× g for 20 min at 4 °C, the collected supernatant served as the crude enzyme solution. A reaction mixture's preparation ensued by adding 2.9 mL of 50 mM K-phosphate buffer (pH 5.5), 1 mL of H_2O_2 (0.6 M), and 1 mL of 50 mM guaiacol to 0.1 mL of the crude enzyme solution (Egley *et al*., 1983). The protein content of the crude enzyme solution, determined at 595 nm with bovine serum albumin, served as the standard using the Bradford assay (Bradford, 1976) by mixing 790 µL of extraction buffer, 10µL of crude enzyme solution, and 200µL of Bradford reagent (Biomatik, Tunisia). Peroxidase activity, determined with guaiacol at 470 nm, reached expression in units per mg of protein (Egley *et al.,* 1983).

Statistical analysis

The two-way analysis of variance (ANOVA) determined water regimes and tested maize hybrid effects and their interactions for all measured traits. The establishment of alphabetical order classes emerged by Tukey's test (α = 5%). Generating a clustered Pearson correlation matrix (α = 5%) proceeded using the trait mean values to study the potential relationship between them. A multiple linear regression analysis also followed. All the performed analyses used the R-Studio environment (R × 64 4.1.2). The reduction and increased rates' calculation for each parameter helped assess the differences in the response to stress imposition.

RESULTS

Water regimes' effect on morphophysiological traits

According to the analysis of variance, the maize (*Z. mays* L.) hybrids, water regimes, and their interactions significantly ($P < 0.001$) affected the shoot length (Table 1). On average, the hybrid Agrister showed an enhanced shoot length compared with the hybrid Sancia under water deficit conditions. By comparing with the control treatment (90% FC), in hybrid Agrister the reduction rate was 6.58% and 6.82% under 75% and 50% FC, respectively, while the increase was 13.9% under 25% FC in shoot length. However, the hybrid Sancia displayed higher reduction rates, reaching 34% under 25% FC (Table 1).

The interaction of maize hybrids and water regimes enunciated a notable effect ($P < 0.001$) on the leaf area (Table 1). The hybrid Agrister displayed the maximum leaf area versus Sancia under 90% FC and other water deficit conditions. The difference in reduction rates of leaf area was remarkable under 50% FC in the hybrids Agrister (3.35%) and Sancia (24.84%). With 25% FC, the water deficit resulted in a leaf area of 60.6 cm^2 in Agrister and 50.9 cm^2 in Sancia. As for the biomass, the water regime had a significant influence ($P < 0.001$) and contributed 82.82% of the total variance (Table 1). The results further showed the biomass decreased with higher water deficit, resulting in an average biomass of 6 g under 25% FC.

The leaf chlorophyll content was markedly ($P < 0.01$) affected by maize hybrids, water regimes, and their interactions, and contributed 9.88% of the total variance (Table 1). The leaf chlorophyll content decreased with increased water deficit conditions. However, the lowest decrease rate was evident in the hybrid Sancia (27.68%) with 25% FC. Overall, the hybrid Agrister displayed higher leaf chlorophyll content values with all the tested water regimes.

The analysis of variance indicated relative water content (RWC) was under the considerable effect ($P < 0.001$) of maize hybrids, water regimes, and their interactions, which contributed 20.61% of the total variance (Table 1). As expected, the RWC decreased with increasing water stress. The difference between the reduction rates of the hybrids Agrister and Sancia was highly substantial, with reduction rates ranging from 1% to 7% in Agrister and from 20% to 74% in the hybrid Sancia. Under 25% FC, the maize hybrid Agrister was able to maintain 66.6% of RWC. Meanwhile, the hybrid Sancia showed only 20.6%. The electrolytic conductivity (EC) significantly ($P < 0.001$) sustained influences from the water regime, contributing 83.21% of the total variance (Table 1). The EC also enhanced with the increasing water deficit, with an average of 30% under 25% FC.

Water regime’s effect on biochemical traits

According to analysis of variance, the H_2O_2, phenolic compounds, catalase, and proline were under the significant ($P < 0.001$) effect of maize hybrids, water regimes, and their interactions, contributing 7.92%, 23%, 33.15%, and 39.32%, respectively to overall variance (Table 2). Inversely, water regimes and the interaction of maize hybrids and water regimes significantly ($P < 0.001$) affected peroxidase, with a share of 93.77% in total variation. Overall, the level of H_2O_2 increased with the rise in water deficit conditions (Figure 1). The hybrid Sancia had higher levels of H_2O_2 than the hybrid Agrister. The highest accumulation of H_2O_2 appeared in the hybrid Sancia (0.27 mmol g^{-1} FW) with 25% FC.

In response to increased water deficit conditions, the maize hybrids differed in the content of phenolic compounds. Under water regimes of 90%, 75%, and 50% FC, the phenolic content was steadily higher in the hybrid Agrister, ranging from 0.26 to 0.27 mg GAE g^{-1} FW. However, under the severe stress (25% FC), the phenolic content became higher in the hybrid Sancia than Agrister (Figure 1). Unlike the hybrid Agrister, the phenolic content rose with enhanced water deficit conditions (25% FC) in the hybrid Sancia to reach the ultimate level of 0.35 mg GAE g^{-1} FW.

As for the peroxidase activity, no significant difference between the maize hybrids Agrister and Sancia occurred. For both hybrids, the peroxidase activity intensified with an increasing water stress, leading to an average of 7.5 mol min^{-1} mg^{-1} P under 25% FC (Figure 1). In both hybrids, the catalase activity also escalated with rising water stress conditions (Figure 1). Besides, and on average,

Table 1. The effects of irrigation water regimes (25%, 50%, 75%, and 90% FC on the shoot length (cm), leaf area (cm^2), biomass (g), relative water content (%), leaf chlorophyll content (%), and electrolytic conductivity (%) of maize hybrids (Agrister and Sancia) under controlled conditions.

Hybrids & water regimes	Shoot length (cm)		Leaf area (cm^2)		Biomass (g)		Relative water content (%)		Leaf chlorophyll content (%)		Electrolytic conductivity (%)	
Hybrid Agrister												
90% (control)	41±0.6	RR%	86.4±0.4	RR%	9.1±0.3	RR%	71.7±1.6	RR%	41.3±0.7	RR%	17.2±0.1	IR%
75%	38.3±0.81	6.58	84.6±0.3	2.08	8.6±0.2	5.49	70.7±0.7	1.39	40.9±0.8	0.96	24.8±0.5	44.18
50%	38.2±0.8	6.82	83.5±0.3	3.35	7.6±0.2	16.4	69.2±0.7	3.48	39.1±0.5	5.32	26.2±0.3	52.32
25%	35.3±0.4	13.9	60.6±0.7	29.86	6.3±0.4	30.76	66.6±2.9	7.11	35.9±0.6	13.07	29.8±1.3	73.25
Hybrid Sancia												
90% (control)	39±0.7		80.9±0.9		8.6±0.3		79.9±0.1		41.9±0.6		17.5±1.1	
75%	35.2±0.7	9.74	73.1±0.7	9.64	8.3±0.2	3.48	63.8±0.8	20.15	37.7±1.1	10.02	23.6±0.7	34.85
50%	30.4±0.9	22.05	60.8±0.7	24.84	7.5±0.4	12.79	48.1±1.2	39.79	34.6±0.7	17.42	26.1±0.4	49.14
25%	25.7±0.8	34.1	50.9±0.3	37.08	6.1±0.7	29.06	20.6±0.7	74.21	30.3±0.9	27.68	30.3±0.7	73.14
Two-way ANOVA												
Hybrids	253.1***		1222***		0.58		2167***		79.7***		0.3 NS	
Water regimes	380.7***		3555***		33.09***		4522***		318.7***		681.4***	
Hybrids × water regimes	80.9***		322***		0.17^{NS}		3187***		43.5***		3.6^{NS}	

The sum square values with significance are shown as NS: Non-significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. ±SD: standard deviation; RR: reduction rate; and IR: increase rate.

Table 2. Analysis of variance of the content of H_2O_2, phenolic compounds, proline, and the catalase and peroxidase measured in two hybrid cultivars (Agrister and Sancia) under four irrigation water regimes (25%, 50%, 75%, and 90% FC).

Source of variation	H_2O_2content	Phenolic compounds	Catalase activity	Peroxidase content	Proline content
Hybrids	0.0115***	0.042***	2.001***	0.000^{NS}	0.004*
Water regimes	0.118***	0.107***	1.566***	179.02***	0.422***
Hybrids × water regimes	0.004***	0.057***	0.332***	1.353**	0.019**

The sum square values with significance are shown as NS: Non-significant, *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$.

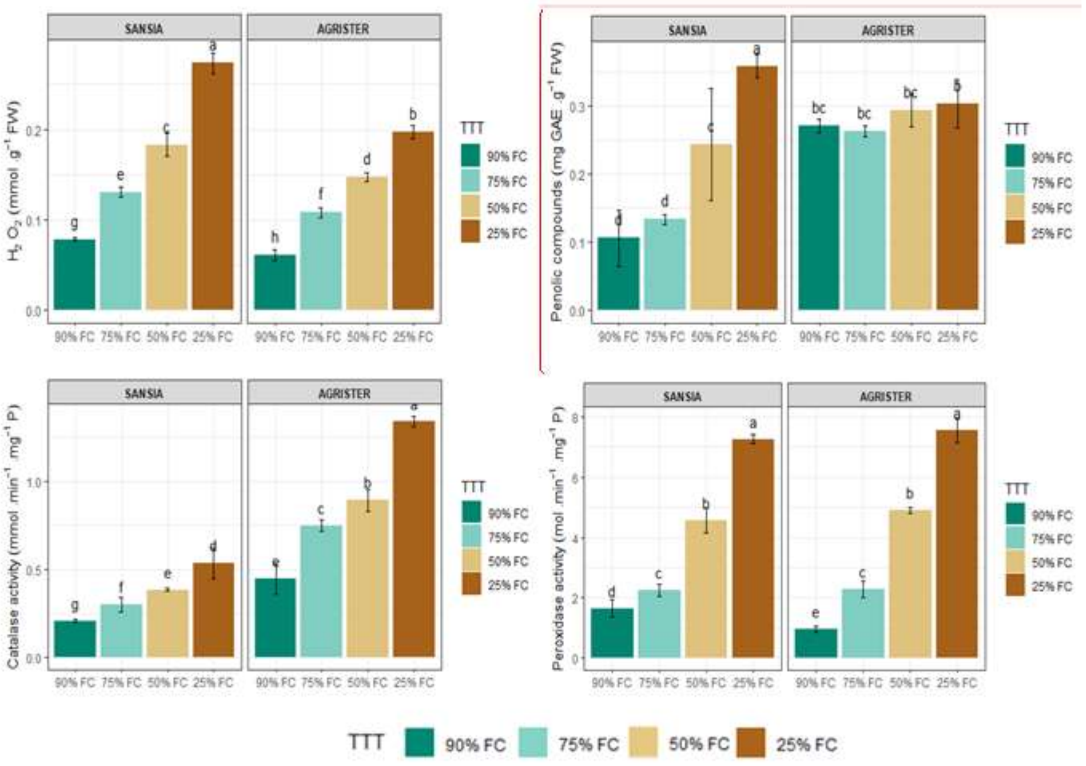
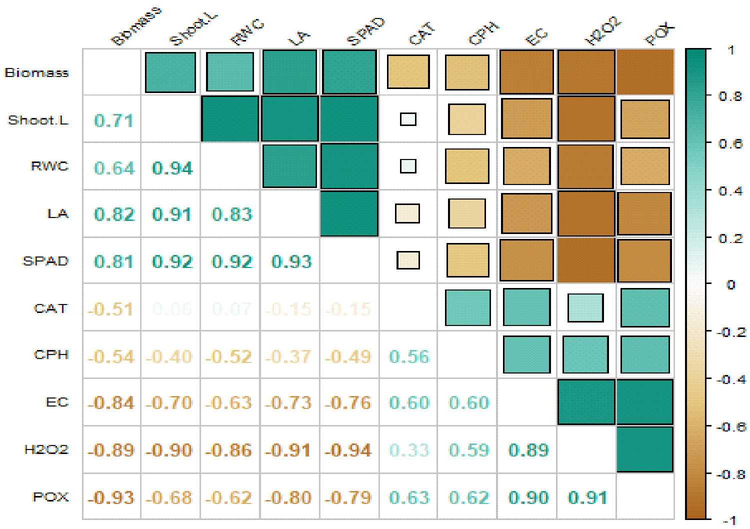


Figure 1. The content in H_2O_2 and phenolics and the activity of peroxidase and catalase of two maize hybrids with four water stress (90%, 75%, 50%, and 25% FC) in controlled conditions at the juvenile growth stage. The alphabetical order classes were established by Tukey's test (α = 5%).



	Biomass	Shoot.L	RWC	LA	SPAD	CAT	CPH	EC	H2O2	POX
Biomass										
Shoot.L	0.71									
RWC	0.64	0.94								
LA	0.82	0.91	0.83							
SPAD	0.81	0.92	0.92	0.93						
CAT	-0.51	0.06	0.07	-0.15	-0.15					
CPH	-0.54	-0.40	-0.52	-0.37	-0.49	0.56				
EC	-0.84	-0.70	-0.63	-0.73	-0.76	0.60	0.60			
H2O2	-0.89	-0.90	-0.86	-0.91	-0.94	0.33	0.59	0.89		
POX	-0.93	-0.68	-0.62	-0.80	-0.79	0.63	0.62	0.90	0.91	

Figure 2. The clustered Pearson correlation matrix between the morpho-physiological and the biochemical traits under water stress conditions in maize. The darker, bigger blue green squares indicate stronger positive correlations. The darker, bigger brown squares indicate stronger negative correlations. ShootL: shoot length, RWC: relative water content, LA: leaf area, CAT: catalase, POX: peroxidase, EC: electrolytic conductivity.

under all the water regimes, the catalase activity was higher in the hybrid Agrister than Sancia. The utmost catalase activity was evident with 25% FC for the hybrid Agrister (1.34 mmol min^{-1} mg^{-1} P).

Correlation analysis

For the morphophysiological and biochemical traits, the Pearson correlation matrix revealed two positively correlated clusters (Figure 2). The first cluster contained the traits of maize biomass, shoot length, relative water content, and leaf chlorophyll content. The second cluster contained the biochemical traits, i.e., catalase, peroxidase, phenolic content, electrolytic conductivity, and H_2O_2. However, the catalase was the only trait showing a weak correlation with other traits. The effect of water stress on the morphophysiological and biochemical traits reached validation by the significant negative correlation between the two positively connected clusters.

DISCUSSION

Maize crop often gains threat from several abiotic constraints, mainly drought, and salinity, considerably the main factors limiting crop growth and grain yield. Many studies showed genotypic variation due to drought stress tolerance. Maize plants employ diverse morphological, physiological, and biochemical mechanisms to mitigate the detrimental effects of drought stress. Thus, the presented study focused on evaluating the two main marketed maize hybrids for water stress tolerance in Tunisia.

As expected, the results signified water deficit conditions induced a significant reduction in general plant growth, depicted by a decrease in shoot length, leaf area, and biomass. This growth reduction also interlinked with a decline in relative water content and chlorophyll content. In fact, plants undergo significant metabolic changes to cope with different stresses through modification in leaf functional traits like leaf area and thickness and stomatal density to regulate their water content, carbon dioxide assimilation, and nutrient absorption capacity (Cai *et al*., 2020; Zhou *et al*., 2020).

Water deficit conditions induced an increase in electrolytic activity, the level of H_2O_2, the peroxidase, and catalase activities. The higher electrolytic activity indicates a leakage of electrons due to a damaged membrane, reflecting the extent of lipid peroxidation. The lipid peroxidation is a consequence of increased production of ROS (H_2O_2, O_2), which can destroy the chloroplast pigments, the membrane lipids and proteins, and the alteration in their structural integrity (Li and Kim, 2022). Plants produce anti-oxidative enzymes, such as, peroxidase and catalase, to prevent and mitigate oxidative damage caused by H_2O_2 (Zandi and Schnug, 2022).

From an overarching viewpoint, the latest study indicated the stress tolerance of the hybrid Agrister compared with Sancia, with their varied response to stress as partially explained by the observed differences in the measured traits. In detail, the highest stress tolerance of the maize hybrid Agrister could be due from the higher shoot length, leaf area, water content, and leaf chlorophyll content, than hybrid Sancia. In hybrid Agrister, the potential of stress tolerance was also characteristic of a lower H_2O_2 content associated with more production of catalase, peroxidase, and phenolic accumulation. Several reports implied antioxidant production promotes defense mechanisms under abiotic stress in *Zea mays* L. (Kumar *et al*., 2015; Ali *et al.*, 2024).

Interestingly, the hybrid Agrister could keep almost a constant level of phenolic compounds independently with the severity of the water stress, suggesting a differential property of this hybrid being able to survive under severe drought conditions. It was a fact that, apart from being ROS scavengers, phenolic compounds can a) absorb light, as photo protectors, thus, limiting the chlorophyll content formation under water deficit conditions, and b) induce leaf rolling, which is an adaptive mechanism to limit water loss (Le-Gall *et al*., 2015; Aliyeva *et al*., 2024). The reported impact of phenolic compounds on chlorophyll content supports the fact that the hybrid Agrister showed greater chlorophyll content depicted by the SPAD values.

Besides its ROS-scavenging capacity, catalase has a key role in photorespiration under abiotic stress conditions. Indeed, the maintenance of CAT activity in maize leaves of drought-stressed plants makes the photorespiration work as energy sink, preventing the over-reduction of the photosynthetic electron transport chain and photo inhibition (Hu *et al*., 2022; Javaid *et al*., 2023). Apart from their H_2O_2 removal capacity, peroxidases perform numerous functions during plant growth and development, including lignin biosynthesis, wound healing, and cell elongation (Zhai *et al*., 2024). Considering the mentioned multiple functions of catalase, peroxidase, and phenolic compounds, it was also relevant to underline how the combined action of phenolics, peroxidase, and catalase has a greater role in drought tolerance of the hybrid Agrister.

H_2O_2 accumulation despite the increasing production of phenolics, peroxidase, and catalase suggested an imbalance between ROS production and the antioxidant defense system. A report submitted this as critical for cell development and plant survival under abiotic stress conditions (Zandi and Schnug, 2022). Under severe water deficit conditions (25% FC), the accumulation of phenolic compounds in the hybrid Sancia, the low activity of catalase and the high level of H_2O_2, indicates its stress sensitivity, leading to chlorophyll degradation and eventually, reduced plant growth.

CONCLUSIONS

(*Zea mays L.)* hybrid Agrister demonstrated superior performance compared with the hybrid Sancia under water deficit conditions, exhibiting greater drought tolerance and better aerial biomass development. This resilience refers to its ability to maintain water and chlorophyll balance while enhancing protection against oxidative stress through the upregulation of catalase, peroxidases, and phenolic compounds. The genetic differences between the two hybrids underscore the potential for breeding drought-resistant maize. Agrister proves as highly recommended for cultivation and further field trials in drought-prone regions of Tunisia.

FUNDING

Financial support provided by PRIMA, a program supported by the European Union under H2020 framework program and PCI2021-121912 funded by MCIN/AEI/10.13039/501100011033.

ACKNOWLEDGMENTS

We Authors are grateful for the exceptional support of the laboratory technicians.

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