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Enhancing Drought Tolerance and Water Productivity of Diverse Maize Hybrids (*Zea mays*) Using Exogenously Applied Biostimulants under Varying Irrigation Levels

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Abstract: Water deficit is a decisive challenge that tremendously affects maize physiological functions and productivity. Hence, it is crucial to ameliorate its tolerance to drought stress, in particular under abrupt climate change and a growing population. The present study aimed to explore the influence of exogenously sprayed moringa seed extract (*Moringa oleifera*) and α-tocopherol on physio-biochemical, morphological, and yield attributes of six diverse maize hybrids under three irrigation levels in poor-fertility sandy soil. The applied irrigation regimes were based on estimated crop evapotranspiration (ET) using the FAO Penman–Monteith equation. A split–split plot arrangement with a randomized complete block design and three replicates was applied for different treatments. Irrigation levels (100% ET, 75% ET and 50% ET) were established in the main plots, while foliar applications (moringa extract and α-tocopherol) were located in subplots and the assessed hybrids (SC162, SC166, SC167, SC168, SC176, and SC178) in subsubplots. Mild (75% ET) and severe (50% ET) drought stress gradually reduced the gas exchange, photosynthetic efficiency, water relations, and yield traits compared with well-watered conditions (100% ET). However, foliar application of moringa seed extract or α-tocopherol was effective in reinforcing maize tolerance to drought stress by enhancing the accumulation of osmoprotectants, improving antioxidant enzymes, and decreasing levels of peroxidation of membrane lipids and electrolyte leakage compared to untreated control. These positive impacts were reflected in boosting yield traits and crop water productivity under water deficit conditions. The physiological and agronomic performance of the assessed maize hybrids considerably varied under water deficit conditions. The hybrids SC168, SC176, and SC178 exhibited the best performance under mild and severe drought conditions compared with the other hybrids. Consequently, the integration of exogenously applied moringa seed extract or α-tocopherol with tolerant maize hybrids such as SC168, SC176, and SC178 is an efficient approach to ameliorating drought tolerance under water-scarce conditions in arid environments.

Keywords: heatmap and hierarchical clustering; Mediterranean region; principal component analysis; physio-biochemical parameters; yield traits

Citation: Mansour, E.; El-Sobky, E.-S.E.A.; Abdul-Hamid, M.I.E.; Abdallah, E.; Zedan, A.M.I.; Serag, A.M.; Silvar, C.; El-Hendawy, S.; Desoky, E.-S.M. Enhancing Drought Tolerance and Water Productivity of Diverse Maize Hybrids (*Zea mays*) Using Exogenously Applied Biostimulants under Varying Irrigation Levels. *Agronomy* **2023**, *13*, 1320. https://doi.org/10.3390/agronomy13051320

Academic Editor: Najeeb Ullah

Received: 19 March 2023
Revised: 15 April 2023
Accepted: 26 April 2023
Published: 8 May 2023

1. Introduction

Maize (*Zea mays* L.) is an expansively grown cereal crop with multiple purposes [1]. It provides food for humans and feed for poultry and animals, as well as being a potential source of biofuel and some industrial products [2]. Its overall cultivated area is approximately 202 × 10^6 hectares, which produces around 1163 × 10^6 tons yearly [3]. Its production should be considerably increased to meet the global rise in its consumption and growing population.

Maize is a highly vulnerable crop to water scarcity [4]. Drought stress is a decisive challenge that tremendously affects maize physiological functions and productivity, mainly in dry environments [4,5]. Water shrinking has detrimental impacts on plant photosynthesis and metabolism [6,7]. Moreover, it causes an increase in the oxidation of cellular components and overproduction of reactive oxygen species (ROS) [8]. Furthermore, water deficit disrupts nutrient absorption and assimilation, as well as carbohydrate metabolism and distribution [9]. In addition, fluctuations in rainfall and increased drought stress are predicted to rise owing to current climate change, mainly in arid environments and water-scarce countries [10]. Hence the constraints of drought stress are more urgent than ever and threaten global food security. Accordingly, it is crucial to assess effective and ecofriendly approaches to attenuate the adverse consequences of water deficit stress on maize.

Foliar applications using different substances (organic or inorganic) are applied to attenuate the adverse impacts of water deficit, therefore enhancing sustainable agriculture and ensuring global food security [11]. Moringa (*Moringa oleifera*) seed extract is a plentiful source of minerals, ascorbic acid, gibberellins, cytokinins, carotenoids, phenols, phytohormones, osmoprotectants, and antioxidants [12]. Moreover, it boosts the mobilization of inorganic solutes such as indole-3-acetic acid, zeatin, potassium, and calcium [13,14]. Furthermore, it enhances the activity of the amylase enzyme and cell elongation, which increases plant growth [15]. Hence, it has abilities to enhance photosynthetic pigments, photosynthetic capacity, antioxidative enzymes, and defense system activity under abiotic stresses. Likewise, α-tocopherol (vitamin E) is a pivotal lipid-soluble antioxidant located in the chloroplast envelope, plastoglobuli, and thylakoid membranes [16]. It has an integral role in the chloroplastic antioxidant network, providing maintained appropriate redox station in chloroplasts, and preserving the thylakoid membrane structure under water stress conditions [17,18]. Moreover, it conserves cell membrane stability, permeability, and photosynthetic machinery from the generated ROS under environmental stresses [19]. It is considered a valuable component of the plant defense system that provides integrity and regular photosynthetic metabolic processes [20]. Therefore, α-tocopherol has a favorable impact on plant productivity under environmental stresses. Consequently, the current study aimed to explore the impact of exogenously sprayed moringa leaf extract and α-tocopherol on physiological, morphological, and yield traits of diverse maize hybrids under different irrigation levels in poor-fertility newly reclaimed sandy soil.

2. Materials and Methods

2.1. Experimental Site and Agricultural Treatments

A field experiment was performed during the two summer seasons of 2020 and 2021 at Elkhatara Experimental Farm, Egypt (30°36′ N, 31°46′ E). The experimental site is described by hot dry weather with no rainfall events during the maize season (Table S1). The average minimum temperature, maximum temperature, growing degree days, and relative humidity were 20.1 °C, 37.6 °C, 576.6 °C, and 50.7%, respectively, during the first season, and 21.0 °C, 38.2 °C, 596.9 °C, and 45.2%, respectively, during the second season. There were no rainfall events during both growing seasons. This site possessed sandy soil throughout, with the following profile: 94.34% sand, 4.55% silt, and 1.11% clay. In both growing seasons, the trails were sown during the recommended period of maize growing in the region, which was the first week of May. Before sowing, 35 kg P ha^{-1} as superphosphate (15.5% P_2O_5) was applied, while 100 kg K ha^{-1} was applied after thinning as potassium sulfate (48% K_2O). Nitrogen fertilizer was performed at a rate of 300 kg N ha^{-1}, with ammonium sulfate (21% N) as fertigation in six splits at 8-day intervals after sowing.

2.2. Plant Material and Irrigation Levels

Six high-yielding single-cross yellow maize hybrids were evaluated in this study. A split–split plot arrangement with a randomized complete block design and three replicates was applied for different treatments. Irrigation levels were established in the main plots, while foliar applications (moringa extract and α-tocopherol) were located in sub-plots, and the assessed hybrids (SC162, SC166, SC167, SC168, SC176, and SC178) in subsub-plots. Each subsubplot consisted of five 4 m long rows, with a 70 cm space between rows and 25 cm space between hills. Each hill was sown with three seeds and thinned to one seedling after full emergence after three weeks. The maize hybrids were evaluated under three irrigation levels based on estimated crop evapotranspiration (ET) utilizing Penman–Monteith equation [21]. Daily evapotranspiration (ET) was determined from meteorological data (including minimum and maximum temperatures, dew point temperature, and wind speed) using the FAO-56 standardized Penman–Monteith equation. The applied well-watered irrigation level (100% ET) was 700 and 765 mm in the first and second seasons, respectively. The well-watered level diminished by 25% and 50% with the application of mild and severe drought stress. The mild drought level was 525 and 574 mm, and the severe drought level was 350 and 383 mm, respectively, in the first and second seasons. Water deficit conditions were induced from seedling establishment (20 days from sowing) up to maturity. The drip irrigation system was performed, employing laterals with a 70 cm space and emitter spacing of 30 cm. A flow meter was used to record the irrigation amount for each irrigation level.

2.3. Preparation and Foliar Application

A total of 200 g of *Moringa oleifera* seeds was blended with 6.75 L of distilled water and 80% ethanol, as outlined by Makkar and Becker [22]. The suspension was mixed utilizing a homogenizer to increase the extracted quantity. The solution was cleaned employing Whatman No.2 filter paper. Moringa (*Moringa oleifera*) seed extract was maintained at −20 °C until utilized. Moringa seed extract was exogenously sprayed at a rate of 5 g L^{-1} and was applied three times during the experiment (20, 35, and 50 days after sowing). A-tocopherol (99.5% pure), which was purchased from Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, was exogenously sprayed at a rate of 1.0 mM, and was also applied three times, at the same times as the moringa seed extract [23,24]. Moringa seed extract and α-tocopherol applications were performed using a pressured spray bottle employing 0.1% Tween 20 as a surface spreader. The distilled water was applied as an untreated control for the foliar applications.

2.4. Measured Parameters

2.4.1. Physiological Parameters

The physiological parameters were measured in the fourth leaf at 60 days after sowing. Total chlorophylls and carotenoid contents were determined utilizing the acetone extract method following Arnon [25]. Photosynthetic efficiency (Fv/Fm) was assessed according to Maxwell and Johnson [26]. The photochemical activity was recorded following Jagendorf [27] with certain modifications in the procedure of Avron [28]. The membrane stability index (MSI) was determined using the following formula: MSI (%) = $(1 - [C_1/C_2]) \times 100$ according to Premachandra et al. [29]. Relative water content (RWC) was determined as outlined by Barrs and Weatherley [30] using the following formula: RWC = $[(FW - DW)/(TW - DW)] \times 100$. Malondialdehyde (MDA) content (μmol/g FW) was determined as described by Heath and Packer [31]. Electrolyte leakage (EL) was measured as presented by Sullivan [32], using the following formula: {EL (%) = $[(EC_b - EC_a)/EC_c] \times 100$}. Proline content (μmol/g DW) was determined, following the method of Bates et al. [33]. Total soluble sugar content (mg/g DW) was determined in leaf ethanol extract, which was reacted with a freshly prepared anthrone reagent, as outlined by Irigoyen et al. [34]. To obtain enzyme extraction, gently cleaned 0.5 g fresh leaf was homogenized in ice-cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA, as described in the method of Vitória et al. [35]. Under cooling, the homogenate was then centrifuged at 15,000× *g* for 15 min. The supernatant was then referred to as the enzyme extract. The activities of catalase (CAT) [36], superoxide dismutase (SOD) [37], and peroxidase (POD) [36] were assayed.

2.4.2. Agronomic Traits

Plant height was determined by recording the length (cm) from ground level to the tassel bottom in 10 plants at each subsubplot at physiological maturity (115 days after sowing). At physiological maturity, three central rows of each subsubplot were collected and sun-dried for two weeks. The biological yield was determined by weighing all these dried plants, and their weight was converted into kg ha^{-1}. The ears of harvested plants were separated, shelled, and weighed to determine grain yield, and converted into kg ha^{-1}. Ten ears were harvested randomly from each subsubplot to record the number of rows and grains per ear. Thousand-grain sets were determined from shelled ears and weighed to record 1000-grain weight. Crop water productivity (kg ha^{-1} m^{-3}) was calculated as the ratio of grain yield or biological yield to evapotranspiration, following Fernández et al. [38].

2.5. Statistical Analysis

R software (version 4.2.1) was employed for statistical analyses. The combined analysis of variance (ANOVA) was applied to explore the differences among the irrigation levels, foliar applications, maize hybrids, and their interactions over the two growing seasons. Combined ANOVA was applied to explore the differences among the irrigation levels, foliar applications, maize hybrids, and their interactions over the two growing seasons using Bartlett's test and Shapiro–Wilk test for the homogeneity of variances and normality distribution of the residuals, respectively. The combined analysis revealed homogenous variances across the two growing seasons for the studied parameter. Accordingly, the data of the two growing seasons were combined. The irrigation levels, foliar applications, and maize hybrids were considered fixed factors while replications, growing seasons, and their interaction were random effects. The differences among the assessed treatments were separated using LSD at a significance level of $p \leq 0.05$. PC-biplot and heatmap were performed using ggplot2 and RcolorBrewer packages implemented in R software. The genetic variability parameters were estimated following Burton and Devane [39]. Path coefficients of grain yield and its related traits were determined according to Dewey and Lu [40].

3. Results

3.1. Photosynthetic Pigments and Photosynthetic Efficiency

Irrigation regimes displayed a significant effect on all parameters of photosynthetic pigments and photosynthetic efficiency (Table 1). The mild and severe water deficit stress gradually declined total chlorophyll content (TChl), carotenoids (Carot), photochemical activity (Photo), net photosynthetic rate (*Pn*), transpiration rate (*Tr*), stomatal conductance (*gs*), and photosynthetic efficiency (Fv/Fm) (Table 1 and Figure 1). Severe drought steeply reduced TChl, Carot, Photo, *Pn*, *Tr*, *gs*, and Fv/Fm by 47.8%, 54.3%, 32.3%, 47.0%, 47.0%, 41.6%, and 25.6%, respectively, compared to well-watered conditions. Moreover, mild drought reduced TChl, Carot, Photo, *Pn*, *Tr*, *gs*, and Fv/Fm by 19.5%, 29.4%, 19.4%, 22.8%, 18.7%, 21.6%, and 14.4%, respectively, compared to well-watered conditions. Nevertheless, exogenously foliar applications of moringa seed extract or *A*-tocopherol considerably enhanced all aforementioned parameters. Moringa seed extract boosted the abovementioned parameters in the same order by 18.3%, 16.6%, 10.8%, 17.8%, 10.8%, 9.4%, and 6.6% compared to untreated treatment (Table 1). Likewise, *A*-tocopherol displayed considerable enhancement by 19.0%, 14.6%, 7.2%, 12.3%, 10.0%, 7.4%, and 4.8% in the same order in comparison with untreated treatment (Table 1). The evaluated hybrids displayed different performances of these parameters. The hybrids SC167, SC168, and SC178 possessed the highest values of all the abovementioned parameters. Under water deficit conditions, these three hybrids in combination with exogenously foliar applications of moringa seed extract or *A*-tocopherol possessed the highest photosynthetic pigments and photosynthetic activities compared to untreated treatment (Figure 1).

Table 1. Influence of irrigation levels and foliar applications on total chlorophyll content, carotenoids, photochemical activity, net photosynthetic rate (*Pn*), transpiration rate (*Tr*), stomatal conductance (gs), and photosynthetic efficiency (Fv/Fm) of six diverse maize hybrids averaged over two growing seasons 2020 and 2021.

Studied Factors		Chlorophyll (mg/g FW)	Carotenoids (mg/g FW)	Photochemical (micromole/ Mgchl/min)	*Pn* (µmol CO_2 /m^2/s)	*Tr* (µmol CO_2 /m^2/s)	*gs* (µmol CO_2 /m^2/s)	Fv/Fm
Irrigation (I)								
Well-watered		2.838 [a]	1.057 [a]	44.23 [a]	13.452 [a]	7.198 [a]	0.668 [a]	0.858 [a]
Mild drought		2.284 [b]	0.746 [b]	35.59 [b]	10.389 [b]	5.849 [b]	0.524 [b]	0.734 [b]
Severe drought		1.481 [c]	0.483 [c]	29.95 [c]	7.136 [c]	3.815 [c]	0.390 [c]	0.638 [c]
Foliar (F)								
Untreated control		1.958 [b]	0.690 [b]	34.52 [c]	9.384 [c]	5.258 [b]	0.499 [b]	0.716 [b]
Moringa seed extract		2.316 [a]	0.805 [a]	38.25 [a]	11.053 [a]	5.823 [a]	0.546 [a]	0.763 [a]
A-tocopherol		2.329 [a]	0.791 [a]	36.99 [b]	10.542 [b]	5.781 [a]	0.536 [a]	0.750 [a]
Hybrid (H)								
SC162		2.138 [c]	0.732 [e]	36.14 [c]	10.169 [c]	5.599 [ab]	0.518 [c]	0.735 [bc]
SC166		2.170 [c]	0.751 [d]	36.33 [c]	10.125 [c]	5.711 [ab]	0.520 [c]	0.737 [bc]
SC167		2.263 [b]	0.783 [c]	36.77 [b]	10.504 [b]	5.751 [ab]	0.539 [ab]	0.751 [ab]
SC168		2.344 [a]	0.816 [a]	37.54 [a]	10.876 [a]	5.836 [a]	0.546 [a]	0.763 [a]
SC176		2.008 [d]	0.700 [f]	35.44 [d]	9.572 [d]	5.389 [b]	0.506 [d]	0.723 [c]
SC178		2.283 [b]	0.791 [b]	37.31 [a]	10.711 [ab]	5.437 [b]	0.533 [b]	0.749 [ab]
ANOVA	**DF**				***p*-value**			
Irrigation (I)	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Foliar (F)	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (H)	5	<0.001	<0.001	<0.001	<0.001	0.024	<0.001	<0.001
I × F	4	<0.001	<0.001	<0.001	<0.001	0.492	0.004	0.03
I × H	10	<0.001	<0.001	<0.001	<0.001	0.014	<0.001	<0.001
F × H	10	<0.001	<0.001	0.001	<0.001	0.359	0.013	0.020
I × F × H	20	<0.001	<0.001	0.001	<0.001	0.053	0.001	0.010

Means followed by different letters under the same factor are different according to LSD test ($p \leq 0.05$). The physiological parameters were recorded at 60 days after sowing.

3.2. Water Relations and Oxidative Stress

Relative water content (RWC) and membrane stability index (MSI) substantially decreased due to water deficit in all evaluated maize hybrids (Table 2 and Figure 2). Severe drought stress diminished RWC by 43.7% and MSI by 51.5% in comparison to well-watered conditions. Mild drought reduced RWC by 20.3 and MSI by 24.7% compared to well-watered conditions. Otherwise, plants spared with moringa seed extract and *A*-tocopherol possessed significantly higher RWC and MSI than untreated plants. Moringa seed extract improved RWC and MSI by 9.4% and 15.2%, respectively, compared to untreated plants. Similarly, *A*-tocopherol exhibited a substantial boost in RWC and MSI by 8.3% and 12.5% compared to untreated plants, respectively (Table 2). The evaluated hybrids exhibited different performances of RWC and MSI. The hybrids SC167, SC168, and SC178 presented the highest values of RWC and MSI under water deficit conditions using both foliar applications (Figure 2).

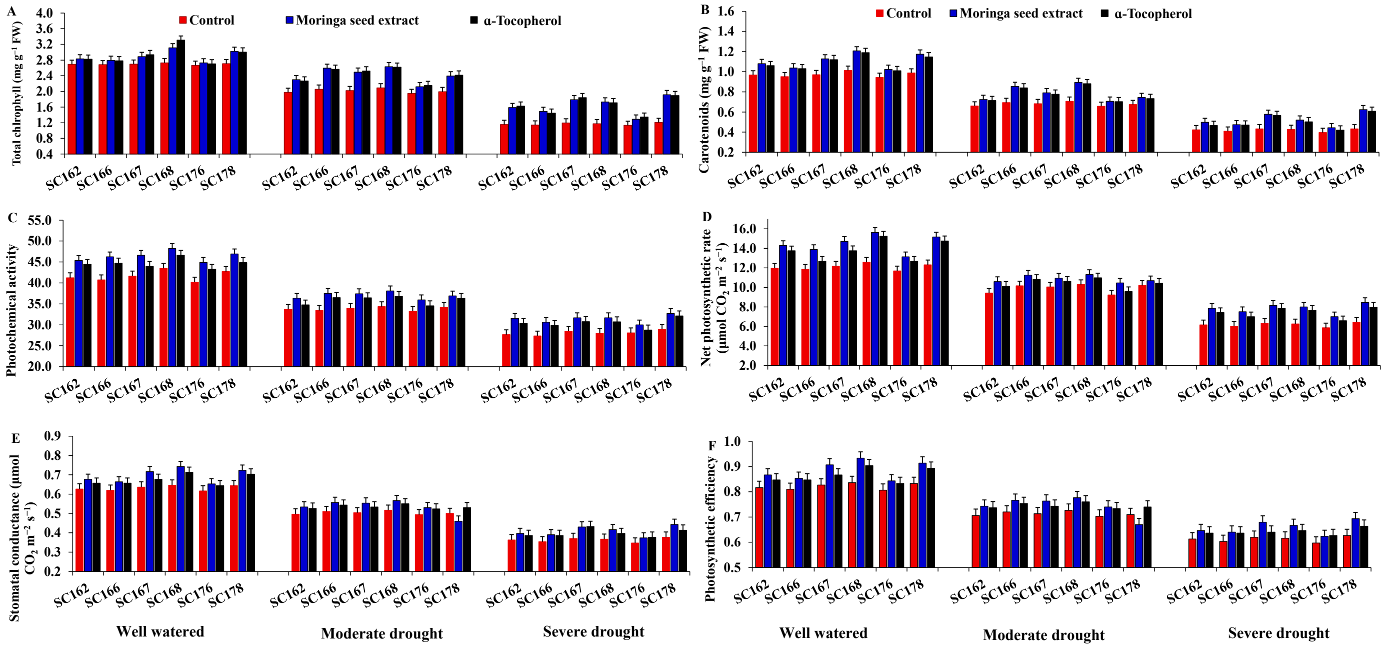

Figure 1. Influence of exogenously sprayed moringa seed extract and *A*-tocopherol on the total chlorophyll content (**A**), carotenoids (**B**), photochemical activity (**C**), net photosynthetic rate (**D**), stomatal conductance (**E**), and photosynthetic efficiency (**F**) of six maize hybrids grown under three irrigation levels over the two growing seasons. The bars on the top of the columns correspond to LSD ($p \leq 0.05$).

Table 2. Influence of irrigation levels and foliar applications on relative water content (RWC, %), membrane stability index (MSI), malondialdehyde (MDA), electrolyte leakage (EL), proline content, soluble sugar (SS), catalase activity (CAT), peroxidase activity (POD), and superoxide dismutase activity (SOD) of six diverse maize hybrids averaged over the two growing seasons 2020 and 2021.

Studied Factors	RWC (%)	MSI (%)	MDA (μmol/g FW)	EL (%)	Proline (μmol/g DW)	SS (mg/g DW)	CAT (unit/mg protein)	POD (unit/mg protein)	SOD (unit/mg protein)
Irrigation (I)									
Well-watered	84.02 a	79.39 a	43.22 c	20.67 c	65.22 c	19.54 c	4.858 c	8.398 c	3.356 c
Mild drought	66.99 b	59.81 b	56.04 b	26.65 b	138.2 b	35.61 b	8.605 b	16.64 b	6.868 b
Severe drought	47.28 c	38.51 c	70.07 a	31.92 a	171.3 a	49.49 a	13.89 a	29.60 a	11.50 a
Foliar (F)									
Untreated control	62.42 b	54.23 c	58.36 a	27.42 a	120.2 c	33.28 c	8.730 b	17.08 c	6.598 b
Moringa seed extract	68.29 a	62.48 a	55.11 b	25.60 c	128.4 a	36.23 a	9.554 a	19.21 a	7.795 a
A-tocopherol	67.57 a	61.00 b	55.86 b	26.22 b	126.1 b	35.13 b	9.072 a	18.35 b	7.329 a
Hybrid (H)									
SC162	64.15 d	57.76 c	57.25 b	26.92 ab	123.5 c	33.89 d	8.788 e	17.85 c	6.796 d
SC166	65.66 c	58.33 c	57.47 b	26.81 b	124.3 bc	34.45 c	8.966 d	17.93 c	7.163 c
SC167	67.58 b	61.09 b	54.60 c	25.99 c	126.3 ab	35.87 b	9.259 c	18.79 b	7.481 b
SC168	68.88 a	62.33 a	54.46 c	25.43 c	128.1 a	36.68 a	9.914 a	19.35 a	7.758 a
SC176	62.62 e	55.03 d	60.25 a	27.40 a	121.1 d	32.27 e	8.248 f	16.31 e	6.472 e
SC178	67.68 b	60.87 b	54.63 c	25.93 c	126.0 b	36.13 b	9.539 b	19.04 b	7.775 a

Table 2. *Cont.*

Studied Factors		RWC (%)	MSI (%)	MDA (µmol/g FW)	EL (%)	Proline (µmol/g DW)	SS (mg/g DW)	CAT (unit/mg protein)	POD (unit/mg protein)	SOD (unit/mg protein)
ANOVA	**DF**					***p*-value**				
Irrigation (I)	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Foliar (F)	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (H)	5	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
I × F	4	<0.001	<0.001	0.003	0.001	<0.001	<0.001	0.050	<0.001	0.006
I × H	10	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
F × H	10	<0.001	<0.001	0.02	0.034	0.136	<0.001	<0.001	0.027	<0.001
I × F × H	20	<0.001	0.001	0.024	<0.001	0.182	<0.001	<0.001	0.001	0.001

Means followed by different letters under the same factor are different according to LSD ($p \leq 0.05$). The physiological parameters were recorded at 60 days after sowing.

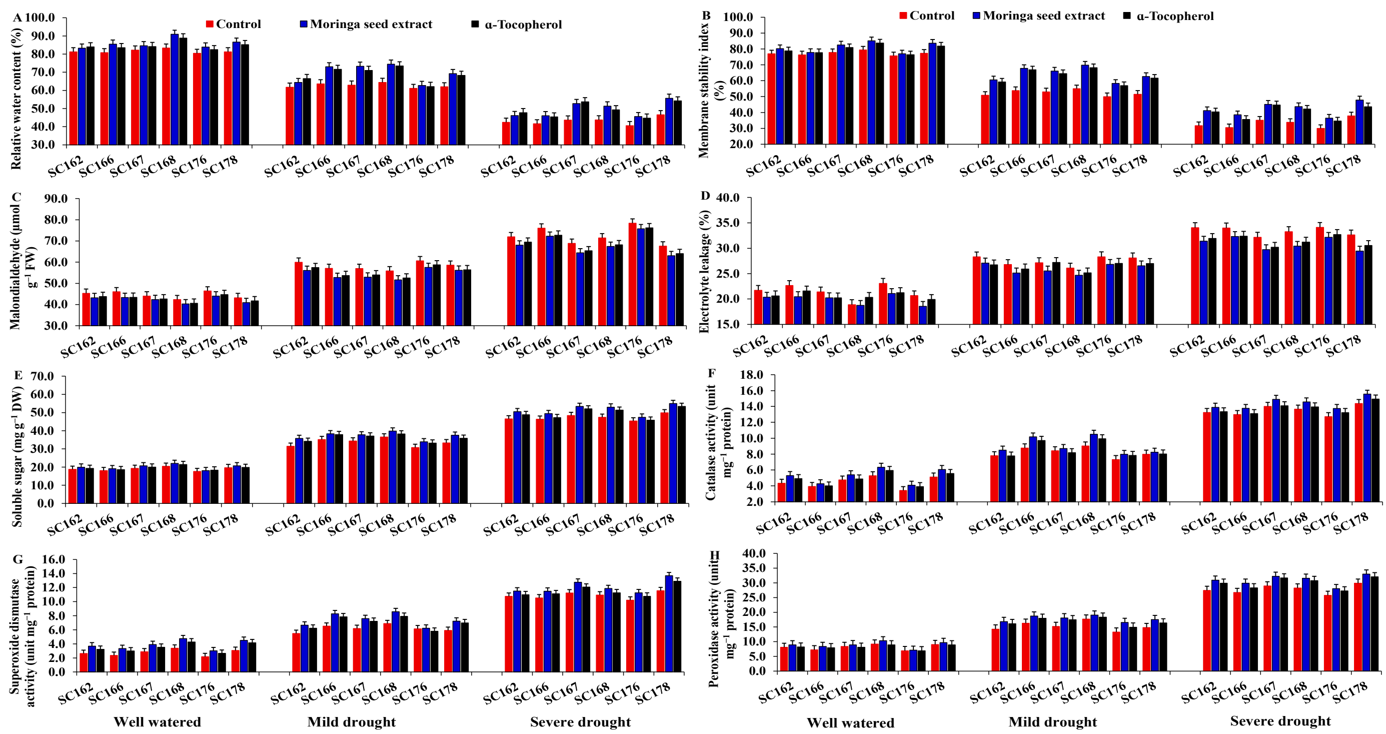

Figure 2. Influence of exogenously sprayed moringa seed extract and *A*-tocopherol on relative water content (**A**), membrane stability index (**B**), malondialdehyde (**C**), electrolyte leakage (**D**), soluble sugar (**E**), catalase activity (**F**), superoxide dismutase activity (G) and peroxidase activity (**H**) of six maize hybrids grown under three irrigation levels over the two growing seasons. The bars on the top of the columns correspond to LSD ($p \leq 0.05$).

Mild and severe drought stress increased malondialdehyde (MDA) by 29.7% and 62.1% and electrolyte leakage (EL) by 29.0% and 54.5%, respectively, compared to well-watered conditions (Table 2). However, the applications of moringa seed extract and *A*-tocopherol significantly reduced the MDA and EL. The evaluated hybrids performed differently under tested irrigation levels and foliar applications. The hybrids SC167, SC168, and SC178 presented the lowest values of MDA and El. The combination of these hybrids with applied foliar application exhibited the best performance under water deficit conditions compared to untreated control (Figure 2).

3.3. Non-Enzymatic and Enzymatic Antioxidants Activities

Mild and severe drought stress elevated proline content (Prol), soluble sugar (SS), catalase activity (CAT), peroxidase activity (POD), and superoxide dismutase activity (SOD)

(Table 2). Severe drought increased MDA, EL, Prol, SS, CAT, POD, and SOD by 162.7%, 153.3%, 186.0%, 252.5%, and 242.7%, respectively, compared to well-watered conditions. Mild drought elevated Prol, SS, CAT, POD, and SOD by 111.9%, 82.2%, 77.1%, 98.1%, and 104.7%, respectively, compared to well-watered conditions. However, the applications of moringa seed extract significantly stimulated Prol, SS, CAT, POD, and SOD by 6.8%, 8.7%, 9.4%, 12.5%, and 18.1% compared to untreated control (Table 2 and Figure 2). Likewise, *A*-tocopherol had a considerable improvement of Prol, SS, CAT, POD, and SOD by 4.9%, 5.6%, 3.9%, 7.4%, and 11.1%, respectively, compared to untreated plants. The assessed hybrids displayed significant differences in the antioxidant activities under assessed irrigation levels and foliar applications. The hybrids SC167, SC168, and SC178 exhibited the highest values of Prol, SS, CAT, POD, and SOD under drought stress, particularly with foliar application of both biostimulants (Figure 2).

3.4. Agronomic Traits

Yield traits were gradually decreased by raising drought levels in all evaluated maize hybrids (Table 3 and Figure 3). Severe drought stress declined plant height (PH), number of rows per ear (Rows/E), number of grains per row (Grains/R), 1000-grain weight (TGW), grain yield (GY), and biological yield (BY) by 12.4%, 7.9%, 22.3%, 15.7%, 40.7%, and 38.5%, respectively, compared with well-watered plants. The exogenously sprayed moringa seed extract and *A*-tocopherol exerted stimulatory impacts on the agronomic traits under both drought conditions. The exogenous application mitigated the inhibitory impacts of water deficit compared to untreated plants. Plants sprayed by both biostimulants possessed significant increases in the evaluated agronomic traits. Moringa seed extract improved PH, Rows/E, Grains/R, TGW, GY, and BY by 3.7%, 4.4%, 4.5%, 4.0%, 9.7%, and 11.1%, respectively, compared with untreated plants (Table 3). Likewise, *A*-tocopherol enhanced the abovementioned agronomic traits in the same order by 3.4%, 4.8%, 4.1%, 3.2%, 9.4%, and 8.4% compared to untreated control. The evaluated hybrids exhibited considerable differences in their agronomic performance under tested irrigation levels and foliar application. The hybrids SC167, SC168, and SC178 presented the highest yield traits. Under water deficit conditions, these three hybrids in combination with the applied foliar application possessed the highest agronomic performance (Figure 3).

Table 3. Influence of irrigation levels, foliar applications, and evaluated maize hybrids on plant height (PH), number of rows/ear (No. rows), number of grains/row (No. grains), 1000-grain weight (TGW), grain yield (GY), biological yield (BY), and crop water productivity of grain yield (CWP_g), or biological yield (CWP_b) of six diverse maize hybrids over the two growing seasons 2020 and 2021.

Studied Factors	PH (cm)	No. Rows	No. Grains	TGW (g)	GY (kg/ha)	BY (kg/ha)	CWP_g (kg/m^3)	CWP_b (kg/m^3)
Irrigation (I)								
Well-watered	243.6 ^{a}	15.28 ^{a}	39.65 ^{a}	254.3 ^{a}	7648 ^{a}	16997 ^{a}	1.053 ^{c}	2.340 ^{c}
Mild drought	235.0 ^{b}	14.84 ^{b}	36.64 ^{b}	233.4 ^{b}	6335 ^{b}	14170 ^{b}	1.163 ^{b}	2.602 ^{b}
Severe drought	213.4 ^{c}	14.07 ^{c}	30.82 ^{c}	214.5 ^{c}	4533 ^{c}	10448 ^{c}	1.248 ^{a}	2.877 ^{a}
Foliar (F)								
Untreated control	225.3 ^{b}	14.29 ^{b}	34.71 ^{b}	228.6 ^{c}	5802 ^{b}	13027 ^{b}	1.073 ^{b}	2.425 ^{b}
Moringa seed extract	233.7 ^{a}	14.92 ^{a}	36.25 ^{a}	237.8 ^{a}	6365 ^{a}	14471 ^{a}	1.200 ^{a}	2.733 ^{a}
A-tocopherol	232.9 ^{a}	14.97 ^{a}	36.15 ^{a}	235.8 ^{b}	6348 ^{a}	14117 ^{a}	1.192 ^{a}	2.662 ^{a}
Hybrid (H)								
SC162	233.4 ^{c}	14.24 ^{f}	35.66 ^{d}	242.6 ^{b}	5829 ^{e}	14329 ^{b}	1.095 ^{b}	2.685 ^{d}
SC166	229.2 ^{d}	14.68 ^{c}	37.47 ^{a}	220.5 ^{e}	6338 ^{c}	13293 ^{e}	1.189 ^{d}	2.500 ^{c}
SC167	221.8 ^{f}	15.22 ^{b}	35.84 ^{c}	237.7 ^{d}	6104 ^{d}	14025 ^{c}	1.151 ^{b}	2.653 ^{c}
SC168	226.6 ^{e}	15.05 ^{a}	36.92 ^{b}	239.2 ^{c}	6582 ^{a}	14950 ^{a}	1.223 ^{a}	2.804 ^{a}
SC176	236.3 ^{b}	14.55 ^{d}	34.03 ^{f}	216.2 ^{f}	5787 ^{f}	12818 ^{d}	1.075 ^{e}	2.397 ^{e}
SC178	236.6 ^{a}	14.62 ^{e}	34.29 ^{e}	248.3 ^{a}	6391 ^{b}	13813 ^{f}	1.195 ^{c}	2.601 ^{b}

Table 3. *Cont.*

Studied Factors		PH (cm)	No. Rows	No. Grains	TGW (g)	GY (kg/ha)	BY (kg/ha)	CWP_g (kg/m^3)	CWP_b (kg/m^3)
ANOVA	**DF**				***p*-value**				
Irrigation (I)	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Foliar (F)	2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hybrid (H)	5	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
I × F	4	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
I × H	10	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
F × H	10	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
I × F × H	20	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means followed by different letters under the same factor are different according to LSD ($p \leq 0.05$). The agronomic traits were recorded at physiological maturity (115 days after sowing).

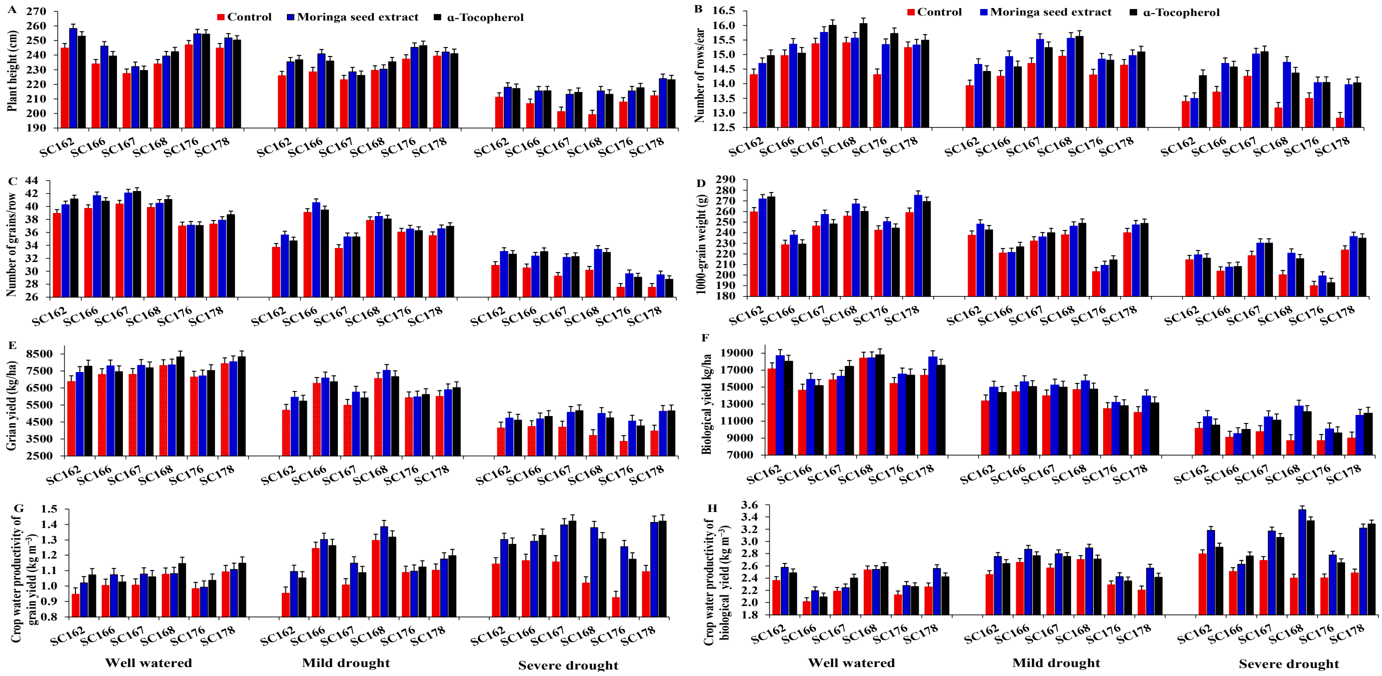

Figure 3. Influence of exogenously sprayed moringa seed extract and *A*-tocopherol on plant height (**A**), number of rows/ear (**B**), number of grains/row (**C**), 1000-grain weight (**D**), grain yield (**E**), biological yield (**F**), crop water productivity of grain yield (**G**), and biological yield (**H**). The bars on the top of the columns correspond to LSD ($p \leq 0.05$).

3.5. Crop Water Productivity (CWP)

Maize plants showed higher crop water productivity of grain yield (CWP_g), and biological yield (CWP_b) under mild and severe drought stress conditions than in well-watered conditions (Table 3 and Figure 3). Exogenously sprayed moringa seed extract and *A*-tocopherol considerably enhanced CWP_g and CWP_b compared with untreated plants. Moringa seed extract improved CWP_g and CWP_b by 11.8% and 12.7%, respectively. Likewise, *A*-tocopherol enhanced CWP_g and CWP_b by 11.1% and 9.8% in the same order. The highest CWP_g and CWP_b under drought stress conditions were assigned for SC167, SC168, and SC178 sprayed with both biostimulants (Figure 3).

3.6. Association among the Studied Parameters and Evaluated Treatments

Multivariate analyses were applied to explore the interrelationship among the assessed treatments and measured parameters under water deficit conditions (Figure 4). The first two principal components explained the maximum variability of approximately 81.49% (69.87% by PC1 and 11.62% by PC2). The PC1 was associated with the foliar applications with the moringa seed extract, and *A*-tocopherol was situated on the opposite positive side. Otherwise, untreated treatments were located on the negative part of PC1. All physiological parameters except MDA and EL exhibited a strong positive association with agronomic traits. The foliar applications of moringa seed extract and *A*-tocopherol were positively associated with photosynthetic pigments, photosynthetic activities, agronomic traits, CWP_g, and CWP_b, particularly on hybrids SC167, SC168, and SC178. On the contrary, these abovementioned parameters were negatively associated with untreated control. The heatmap and hierarchical clustering based on the studied physiological and agronomic parameters divided foliar applications and maize hybrids under drought stress into different clusters (Figure 5). The foliar applications were the principal dividing factor of the main clusters. Untreated control had the lowest values (red values), while moringa seed extract and *A*-tocopherol exhibited the highest values for most studied parameters (depicted in blue). The maize hybrids displayed different responses under drought stress, but in general, SC168, SC167, and SC178 sprayed with both biostimulants displayed the highest values of most evaluated parameters.

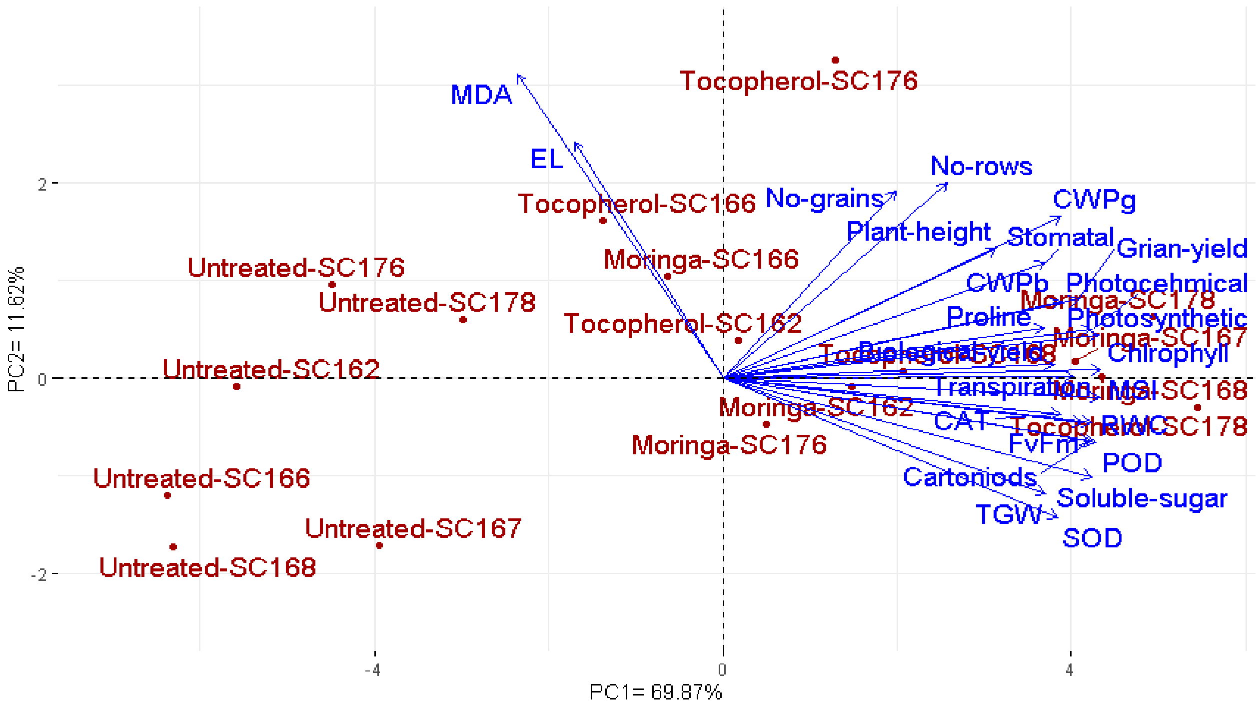

Figure 4. Principal component biplot for the applied foliar applications and assessed maize hybrids based on the studied parameters under drought stress over two growing seasons. MDA is malondialdehyde, EL is electrolyte leakage, FvFm is photosynthetic efficiency, SOD is superoxide dismutase activity, CAT is catalase activity, POD is peroxidase activity, Stomatal is stomatal conductance, Photochemical is photochemical activity, Photosynthetic is net photosynthetic rate, MSI is membrane stability index, RWC is relative water content, No-rows is number of rows per ear, TGW is 1000-grain weight, No-grains is number of grains per row, CWPg is crop water productivity of grain yield, CWPb is crop water productivity of biological yield.

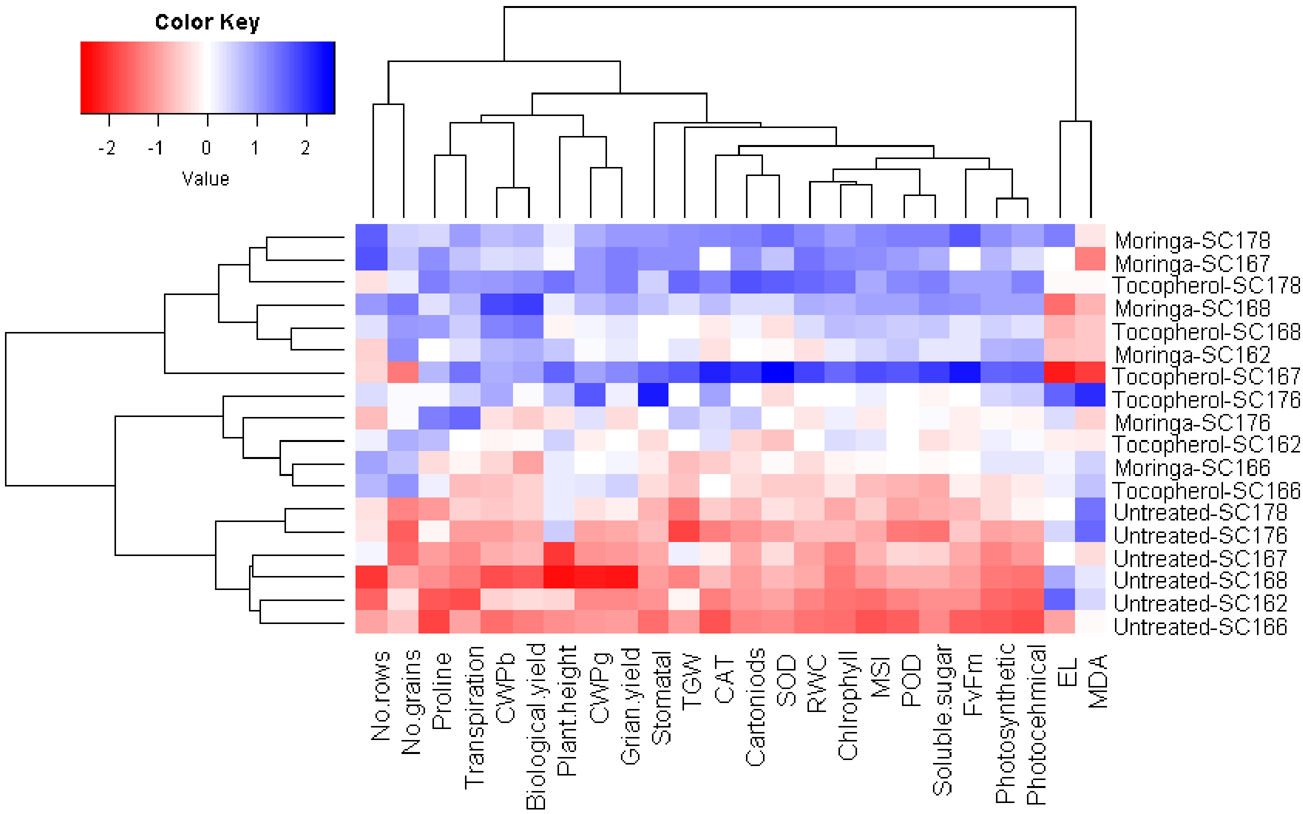

Figure 5. Heatmap and hierarchical clustering dividing the evaluated foliar applications and maize hybrids under drought stress into different clusters based on the assessed physiological and agronomic parameters. Blue color and red color indicate high and low values for the corresponding parameters, respectively. MDA is malondialdehyde, EL is electrolyte leakage, FvFm is photosynthetic efficiency, SOD is su-peroxide dismutase activity, CAT is catalase activity, POD is peroxidase activity, Stomatal is sto-matal conductance, Photochemical is photochemical activity, Photosynthetic is net photosynthetic rate, MSI is membrane stability index, RWC is relative water content, No-rows is number of rows per ear, TGW is 1000-grain weight, No-grains is number of grains per row, CWPg is crop water productivity of grain yield, CWPb is crop water productivity of biological yield.

3.7. Genetic Variability and Path Analysis for Agronomic Traits

Genetic variability parameters for agronomic traits of evaluated maize hybrids under drought stress were estimated to explore the magnitude of variation for these traits (Table 4). The phenotypic coefficient of variation (PCV) was closer to their related genotypic one (GCV) for number of rows per ear, plant height, number of grains per ear, and 1000-grain weight, whereas PCV values were divergent from their corresponding GCV in grain and biological yields. The heritability estimates were moderately low for grain and biological yields, while moderate values were determined for 1000-grain weight and number of grains per ear. However, high heritability was recorded for number of rows per ear and plant height. Furthermore, path analysis was performed to determine direct and indirect effects of agronomic traits on grain yield in maize under drought stress (Table 5). The highest positive direct effect on grain yield was provided by 1000-grain weight, followed by number of rows per ear and biological yield. The highest positive indirect effects were detected for crop water productivity of biological yield, crop water productivity of grain yield, and biological yield via 1000-grain weight, respectively. Number of grains per row also had a cognizable indirect effect via number of rows per ear.

Table 4. Genetic variability parameters for the studied agronomic traits in maize hybrids under drought stress.

Parameter	Plant Height	No of Rows/Ear	No of Grains /row	1000-Grain Weight	Biological Yield	Grain Yield
Genotypic variance	18.05	0.187	2.551	328.1	901,842	165,034
Environmental variance	3.560	0.020	1.071	119.34	865,676	149,352
Phenotypic variance	21.61	0.207	3.622	447.4	1,767,518	314,386
Genotypic coefficient of variance	1.976	3.057	5.092	7.478	8.895	8.583
Phenotypic coefficient of variance	2.162	3.216	6.067	8.733	12.45	11.85
Heritability (broad sense)	83.53	90.34	70.43	73.33	51.02	52.49

Table 5. Direct and indirect effect of agronomic traits on grain yield in maize under drought stress.

Trait	Plant Height	No. of Rows/Ear	No. Grains /Row	1000-Grain Weight	Biological Yield	CWPb	CWPg
Plant height	**0.088**	−0.280	−0.001	0.417	0.133	0.033	−0.087
No. of rows per ear	0.050	**0.488**	0.001	−0.202	−0.081	0.106	0.053
No. grains per row	0.062	0.308	**0.002**	−0.204	−0.121	0.044	0.079
1000-grain weight	−0.044	−0.119	−0.0004	**0.830**	−0.260	0.203	0.170
Biological yield	0.029	0.098	0.0005	0.536	**0.403**	0.184	0.263
Crop water productivity of biological yield (CWPb)	−0.011	0.197	0.0003	0.647	−0.284	**0.265**	0.185
Crop water productivity of grain yield (CWPg)	0.029	0.099	0.0005	0.536	−0.403	0.183	**0.261**

4. Discussion

Maize is a highly sensitive cereal crop to water deficit, and its productivity is severely limited by drought stress [4]. Appropriately, the exogenous application of promoting substances is an advantageous approach for enhancing maize drought tolerance and boosting its productivity under drought stress [41,42]. In the current study, exogenously sprayed moringa seed extract and α-tocopherol were applied to assess their impacts on the physiological, morphological, and agronomic performance of six diverse maize hybrids under three varying irrigation levels over two years of field trials. The combined analysis of variance indicated homogenous variances across the two growing seasons for studied parameters, and the yearly differences were insignificant. The studied irrigation regimes, applied biostimulants, assessed maize hybrids, and their interactions exhibited significant effects on most measured parameters. The typical summer growing season in Egypt is described by hot dry weather with no rainfall events. Accordingly, the applied mild and severe drought conditions gradually decreased the physiological activities of maize plants, including membrane stability, relative water content, photosynthetic pigments, leaf gas exchange, and photosynthetic efficiency, while malondialdehyde, electrolyte leakage, soluble sugar, proline content, and antioxidant enzymes increased compared with well-watered conditions. Appreciably, measurements of photosynthetic pigments and photosynthetic efficiency are valuable indices for exploring drought tolerance in maize [43,44]. The results revealed that the applied exogenously moringa seed extract and α-tocopherol improved maize drought tolerance by boosting photosynthetic pigments and gas exchange (stomatal conductance, net photosynthetic rate, and transpiration rate) in comparison with untreated plants under drought stress conditions.

The analysis of moringa seed extract alluded that it contains various macronutrients and microelements, antioxidants such as salicylic acid, ascorbic acid, proline, soluble sugar, as well as phytohormones such as gibberellins, indol-3-acetic acid, and cytokinin (Table S2). In this context, Yasmeen et al. [45] elucidated that moringa extract inhibits early leaf senescence and provides more leaf area, which increases photosynthetic pigments. Consequently, staying green enhances leaf photosynthesis and increases sink capacity and the photoassimilates [46,47]. Accordingly, moringa seed extract can be employed as a plant biostimulant to enhance photosynthetic pigments and gas exchange under abiotic stresses. Moreover, Elrys et al. [13] and Desoky et al. [48] demonstrated that the foliar application of biostimulants such as moringa seed extract plays a valuable role in enhancing tolerance against environmental stresses by boosting photosynthetic pigments and photosynthetic activity. Likewise, α-tocopherol also stimulates numerous physiological processes under drought stress such as cell differentiation, metabolism process, nutrient availability, photosynthetic activities, and growth regulation [19]. Moreover, it is a vital antioxidant that has a crucial role in deactivating ROS, scavenging lipid peroxidation in thylakoid membranes, and the neutralization of singlet oxygen and superoxide radicals in plant cells [20]. Consequently, its foliar application boosted photosynthetic pigments, gas exchange, and photosynthetic metabolic processes under water deficit conditions. In this context, Ali et al. [19], Lalarukh et al. [49], and El-Beltagi et al. [50] depicted that the application of α-tocopherol considerably promoted the chlorophyll and carotenoid concentration and scavenged free radicals and peroxides, accordingly reinforcing the plants' ability to diminish the destruction caused by ROS.

Plant water status is extremely sensitive to drought stress and is a commonly explored plant response to water scarcity conditions. Water shortage declines root hydraulic conductivity and reduces water movement to shoots, which causes a lowered leaf water content and the closure of stomata to preserve their water status [51]. A reduction in leaf water content and membrane stability causes toxic impacts, metabolic alterations, and growth inhibition [52]. The results manifested that the foliar-applied moringa seed extract or α-tocopherol significantly increased water relation, i.e., RWC and MSI, compared to untreated plants in all evaluated hybrids grown under water deficit conditions. In the same way, Basu et al. [53] manifested that the exogenously applied moringa extract enhanced RWC and MSI under water shortage. Likewise, Elrys et al. [13] disclosed that moringa extract application maintained the RWC and MSI of the subjected plants to drought stress by elevating water, pressure, and osmotic potentials. Similarly, Ali et al. [54], Ali et al. [49], and Shah et al. [20] suggested that the application of α-tocopherol, substantially boosted RWC and MSI, as well as sustained cell turgid and membrane integrity under water-scarce conditions.

The peroxidation of membrane lipids and electrolyte leakage could be signs of oxidative damage, and are often used as indicators for determining the damage extent under different stresses [55]. Decreasing malondialdehyde (MDA) and electrolyte leakage (EL) levels allude to a low level of impairment caused by drought stress, which reflects the enhancement of drought tolerance in stressed plants [56]. The obtained results pointed out that exogenously sprayed moringa seed extract or α-tocopherol caused a considerable reduction in the contents of MDA and EL under water deficit conditions compared to untreated control. Reductions in MDA and EL due to the application of moringa seed extract or α-tocopherol demonstrated their role in maintaining the structure and stability of plasma membranes under water-scarce conditions [57,58]. Correspondingly, Szarka et al. [59], Jungklang et al. [60], and Elrys et al. [13] deduced that the application of moringa seed extract and α-tocopherol reduced the ionic leakage and lipid peroxidation compared to untreated plants under drought stress. Hence, their applications mitigated the induced detrimental effects of water shrinking by diminishing the levels of ionic leakage and lipid peroxidation, as well as enhanced membrane integrity, osmolyte accumulation, and different metabolites.

Soluble sugar and free proline contribute to osmotic alteration and can directly or indirectly adjust the storage functions, gene expression in the metabolic processes, and defense system under abiotic stresses [61,62]. Moreover, they detoxify the generated ROS in plant cells and react promptly with hydroxyl radicals or physically quench singlet oxygen [63,64]. Therefore, elevating the plant content of proline and soluble sugar promotes the antioxidant system and mitigates injurious impacts caused by water scarcity. The application of moringa seed extract or α-tocopherol elevated soluble sugar and proline content in all evaluated maize hybrids, and superior concentrations were detected under severe drought stress. Correspondingly, Desoky et al. [15] and Howladar [65] disclosed that exogenously applied moringa extract increased the content of soluble sugar, proline, amino acid, and antioxidants under abiotic stresses. Hence, the exogenously applied biostimulants regulated the innate mechanisms and enhanced the non-enzymatic antioxidant defense system [14,66]. Likewise, Shah et al. [20] demonstrated that α-tocopherol application induced physiological and biochemical changes, including increasing the content of free proline and soluble sugar, as well as the antioxidant activity. Furthermore, Orabi and Abdelhamid [67] and Hemida et al. [17] deduced a significant improvement in proline content by the foliar-applied α-tocopherol, which was reflected in regulating plant metabolism, stimulating the defense system, mitigating stress injuries, and enhancing the plant tolerance to environmental stress.

There is a robust association between tolerance to environmental stresses and enhancing antioxidant activities [68,69]. Appreciably, the activities of antioxidant enzymes raised in the plants to cope with drought stress to eliminate reactive oxygen species. The activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) increased under mild and severe drought stresses. Moreover, the application of moringa seed extract and α-tocopherol markedly enhanced the antioxidant system by promoting CAT, POD, and SOD activity compared with untreated control under water-scarce conditions. Enhancement of the enzyme activities stimulates the capacity of defense against oxidative damage induced by drought stress in maize plants, and accordingly improves drought tolerance. In this context, Elrys et al. [13], Shah et al. [20], Yasmeen et al. [45], Lalarukh et al. [49], and Orabi and Abdelhamid [67] disclosed that the application of moringa extract and α-tocopherol elevated the antioxidant enzymes against oxidative stress.

With the knowledge of abrupt changes in climate, identifying drought-tolerant genotypes is one of the crucial approaches to alleviating detrimental impacts induced by drought stress, particularly in water-scarce areas [70–72]. In this study, physiological, morphological, and agronomic attributes were employed to assess the response of six maize hybrids to different irrigation levels. Remarkably, the evaluated maize hybrids demonstrated significant differences in their responses to water deficit conditions. The assessed hybrids exhibited substantial alterations in the photosynthetic attributes, gas exchange, and enzyme activities under different irrigation levels. The hybrid's pattern altered more under drought than well-watered conditions. The highest physiological parameters under drought conditions were assigned for SC167, SC168, and SC178. These hybrids proved to be drought-tolerant by enhancing photosynthetic efficiency, gas exchange, water relation, and enzymatic antioxidants. These enhancements were reflected in increasing their agronomic traits compared to the other ones under drought stress conditions, particularly under the exogenous application of both applied biostimulants.

The interaction among the studied irrigation regimes, applied biostimulants, and assessed maize hybrids exhibited significant effects on most measured parameters. Generally, under water deficit conditions, the hybrids SC167, SC168, and SC178 in combination with exogenously foliar applications of moringa seed extract or *A*-tocopherol possessed the highest photosynthetic pigments, photosynthetic activities, enzymatic and non-enzymatic antioxidants, maize growth, production, and crop water productivity compared to untreated treatment. The biplot of principal components and the heatmap and hierarchical clustering are useful statistical approaches to exploring the interrelationships between the studied factors (as interaction effect of three factors) and the evaluated parameters [73,74]. These models were applied to consider the relationship among studied factors and evaluated parameters in numerous published reports [19,73,75–77]. The results of the PCA biplot and heatmap strengthened the positive influences of both studied bio-stimulants; moringa seed extract and *A*-tocopherol on all evaluated parameters under all studied irrigation regimes. The PCA biplot and heatmap exhibited that photosynthetic pigments, photosynthetic activities, agronomic traits, CWP_s, and CWP_{ab} were positively associated with foliar-applied moringa seed extract and *A*-tocopherol, particularly with the hybrids SC167, SC168, and SC178 under water deficit conditions. These results corroborated that the exogenous applications of both biostimulants enhanced the physiological parameters, yield traits, and crop water productivity of maize, in particular under drought stress. Subsequently, the exogenously applied moringa seed extract or *A*-tocopherol could be a valuable approach to promoting maize yield and crop water productivity. Number of rows per ear and 1000-grain weight exhibited positive direct effects on grain yield. This indicated that any increase in one of these traits would directly contribute to enhancing grain yield. Therefore, selection for these traits is effective for improving the grain yield of maize under drought stress, as they displayed good heritability values. By analogy, the physiological parameters representing very close vectors with an acute angle with grain yield displayed great importance for indirect selection under water deficit conditions.

5. Conclusions

The exogenous application of moringa extract or α-tocopherol is an efficient approach to mitigate the negative impacts induced by water deficit through boosting photosynthetic pigments, photosynthetic activities, and enzymatic and non-enzymatic antioxidants. These enhancements were reflected in the improved maize growth, production, and crop water productivity of different maize hybrids. The evaluated maize hybrids displayed considerable genetic differences under studied irrigation regimes. The hybrids SC167, SC168, and SC178 exhibited more tolerance to water deficit conditions based on physiological parameters, yield traits, and crop water productivity. Hence, foliar-supplied moringa seed extract or α-tocopherol could be employed as an efficacious approach to enhance drought tolerance of promising tolerant maize hybrids under water scarcity conditions. Number of rows per ear, 1000-grain weight, and other physiological parameters displayed positive associations with grain yield, and could be exploited for indirect selection in breeding programs to drought tolerance.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13051320/s1, Table S1. Monthly average minimum (Min) and maximum (Max) temperatures, growing degree days (GDD), relative humidity (RH), and total rainfall (Rain) in 2020 and 2021 growing seasons as well as 28-yr monthly averages (1994–2021). Table S2. Chemical analysis of the Moringa seed extract.

Author Contributions: Conceptualization, E.M., E.-S.E.A.E.-S., M.I.E.A.-H., E.A. and E.-S.M.D.; methodology, E.M., E.-S.E.A.E.-S., M.I.E.A.-H., E.A., A.M.I.Z., A.M.S. and E.-S.M.D.; software, E.M., C.S., S.E.-H., E.M., E.-S.E.A.E.-S., M.I.E.A.-H., E.A., A.M.I.Z., A.M.S. and E.-S.M.D.; formal analysis; E.M., E.-S.E.A.E.-S., M.I.E.A.-H., E.A., A.M.I.Z., A.M.S. and E.-S.M.D.; resources, C.S. and S.E.-H.; writing—original draft preparation, E.M., C.S., S.E.-H. and E.-S.M.D.; writing—review and editing, E.M., C.S., S.E.-H. and E.-S.M.D.; funding acquisition, C.S. and S.E.-H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Researchers Supporting Project number (RSPD-2023R730), King Saud University, Riyadh, Saudi Arabia.

Data Availability Statement: The data presented in this study are available upon request from the corresponding authors.

Acknowledgments: The authors extend their appreciation to the Researchers Supporting Project number (RSPD-2023R730), King Saud University, Riyadh, Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

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