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Enhancing Water Limitation Tolerance of Tomato Plants Grown Under Water-limited Irrigation Regime by Maize Grain Embryos Extract Enriched with Some Bio-stimulant (MEEst)

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ABSTRACT

This study was conducted on the preventive measure of spraying tomato plants with a natural extract derived from maize grain embryos that is enriched with gibberellic acid (GA_3), ascorbate (AsA), and selenium (Se) (MEEst) to guard against damage caused by water-limited stress (WL). A fully randomized factorial design was used to arrange the treatment with 7.5 or 15.0% MEEst and WL treatment (60% of soil relative water content; SRWC) versus 100% of SRWC (control). Some physio-biochemical compounds and the activities of some antioxidant enzymes were measured. Significant reductions in hormonal balance (indole acetic acid (IAA), GA_3, zeatin-type cytokinins (Zeatin-CK), and cytokinins (CKs), as well as higher abscisic acid (ABA)) were observed under WL conditionds. Also, enzyme activities (superoxide dismutase, ascorbate peroxidase, catalase, and glutathione reductase) were enhanced, low molecular mass antioxidant compounds (ascorbic acid (AsA), glutathione (GSH), and α-tocopherol (α-TOC)), and osmo-protectants were increased, as a result of increasing reactive oxygen species; hydrogen peroxide radicals and superoxide. On the other hand, treatment with 15.0% MEEst significantly improved hormonal balance (IAA, GA_3, Zeatin-CK, and CKs by 37%, 53%, 69%, and 59%, respectively, while ABA decreased by 47%). Low molecular mass antioxidant compounds (AsA, GSH, and α-TOC) increased by 32%, 47%, and 48%, respectively. Additionally, osmo-protectants (free proline, soluble sugars, glycine betaine, and total soluble protein contents) were increased by 51%, 43%, 23%, and 106%, respectively under WL conditions. Finally, 15.0% MEEst concentration showed achievement over treatment with 7.5% MEEst.

1. Introduction

Throughout history, plants have played a crucial role in human survival by providing 82% of the world's total food production that is derived from land resources. One of the most productive vegetable crops in the world is the tomato (*Solanum lycopersicum* L., a member of the *Solanaceae* family) [1]. In addition to being consumed, it is utilized as a model plant for research purposes to examine its physiology, genetics, and productivity in a variety of environmental settings [2]. Changes in the global climate have resulted in notable increases in temperature and fluctuations in precipitation. Climate change therefore makes water-limited stress (WL) worse and increases the likelihood and intensity of its occurrence [3,4]. Inadequate water absorption is the root cause of WL, a condition that impacts crop development, gene expression, distribution, harvest, and quality by preventing the absorption of extra nutrients [5]. In a similar vein, it severely restricted crop productivity worldwide by causing physiological, biochemical, and morphological damages [6].

Egypt is one of the countries that suffer from WL problems [7]. WL has been happening more frequently and severely recently as a result of the effects of global climate change, and it poses varying degrees of threat to most countries and regions [8]. Water resource management has become a global concern [9]. Torres-Ruiz et al.[10] stated that WL can significantly affect the biochemistry and physiology of a plant. It may also have an impact on the plant's water usage. Consequently, more research into the responses and adaptations of plants to water-logging stresses is needed to develop WL-tolerant, extremely water-efficient plant systems for agricultural use.

Water limitation induces the production of reactive oxygen species (ROS), such as $O_2^{\bullet-}$ and H_2O_2. While they are produced during regular metabolic processes such as photosynthesis and cellular respiration, several enzymes and antioxidants that have been developed specifically for this purpose

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typically control their levels [11]. Additionally, ROS causes oxidative damage and negatively affect the synthesis of important molecules such as proteins, sugars, lipids, and nucleic acids [12].

Antioxidant defense systems against WL include enzymatic antioxidants such as glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) and non-enzymatic antioxidants such as glutathione, ascorbate, α-tocopherol, proline, carotenoids, and flavonoids [13]. Increased accumulation of osmosis-related substances, such as soluble protein, soluble sugars, glycine betaine, proline, etc., supports the antioxidant defense system and helps shield cellular structures from ROS [13]. By improving osmotic adjustment and the antioxidant defense system to support WL tolerance, the osmosis-related compounds play critical roles in the detoxification of WL-induced ROS [4]. To counteract this detrimental impact, which is getting worse every day, more research is required to adopt and develop technological solutions that producers and farmers can apply to stressed plants to prevent losses in agricultural production [11]. The components of plants' endogenous antioxidant system prevent them from withstanding extreme stress most of the time. Therefore, in order to increase plants' resistance to WL, exogenous applications (like antioxidants and plant extracts) should be used [11,14].

Many physiological processes can be improved by a wide range of bioactive substances referred to as biostimulants, which in turn encourage the growth and development of plants [15]. Additionally, it has been demonstrated that using plant-based natural extracts from dry bean seeds, maize grains, and *Moringa oleifera* leaves as foliar sprays or seed presoaking solutions enhances plant performance in both normal and stressful situations [16,17].

One of the main strategies for WL relief is the use of plant extracts, which are rich in bioactive stimulators that are essential for plant growth under stress [18,19]. Recently, maize grain embryos-derived natural extracts (MEE) have been used for seed and foliar treatments and proven to ameliorate plant growth and its outputs under WL [11,20]. MEE is categorized as an organic biostimulant that promotes plants' resilience to harsh environments due to its abundance in cytokinins (especially zeatin-type cytokinins), auxins (especially indole-3-acetic acid), gibberellins, antioxidants, and ameliorating essential nutrients for morphological, biochemical, and physiological processes [21].

As a crucial substrate of APX, an enzyme involved in the ascorbate–glutathione pathway, ascorbic acid serves as plants' first line of defense against oxidative stress by removing several free radicals, including $HO^{\bullet}$, H_2O_2, and $O_2^{\bullet-}$ [22]. Gibberellin mechanisms involved elevated antioxidant enzyme activity, inhibition of ribonuclease and polyphenol oxidase activities, promoted biosynthesis of proteins, and improved levels of reducing sugars [23]. Selenium is believed to be beneficial to higher plants as it improves antioxidant metabolism, secondary metabolites, photosynthesis, and carbohydrates amount [24].

The current study set out to investigate the hypothesis that the application of MEEst as foliar nourishment for tomatoes can provide an advantage to overcome WL by improving plant growth and productivity through its favorable effect on the accumulation of organic solutes, ionic homeostasis, enzymatic and non-enzymatic antioxidant defense machinery, and plant hormonal balance. Hence, the objective of this investigation was to describe the mechanisms regarding the mitigation of WL detrimental impacts in MEE-treated plants. Moreover, using MEEst as an exogenous plant growth enhancer could help to emerge a new dimension into the use of the exogenous applications on stressed plants, as their productivity may be improved by MEE to contribute to global food security.

2. Materials and Methods

2.1. Tomato transplant source and preparation for planting

Thirty-five-day-old tomato (*Solanum lycopersicum* L.) transplants (cultivar 023) were obtained from the Nurseries of Agriculture Ministry, Cairo, Egypt. After the transplants were sorted to ensure their authenticity and symmetry, they were transplanted. Two irrigation treatments (100% and 60% of SRWC) were chosen based on our preliminary pot study, in which 60% of SRWC weakened plant growth. Fifteen days post transplanting, the irrigation treatments, and MEEst (with two concentrations) were applied as foliar spraying in the early morning. Fifteen and thirty days after the first spraying, the second and third foliar sprays were implemented. To guarantee the best penetration, a few drops of Tween-20 were added to the spraying solution to act as a surfactant. Based on tomato growth results in preliminary trials (Table 1), the MEEst of 7.5 and 15.0% were chosen for this study.

2.2. Growth conditions and planning treatments

A pot experiment was conducted at the experimental farm of the Faculty of Agriculture, South East Fayoum (29° 17′N; 30° 53′E), Egypt. Transplanting was performed in August 2022.Twenty plastic pots, each measuring thirty centimeters in diameter and depth, were used in this investigation. A 9.5 kg medium with 90.0% pure sand, 6.5% compost, 3.0% vermicompost, and 0.5% humic acid were added [25]. Prior to this, a commercial acid was used to thoroughly clean the sand of all ions, and SD-H_2O was used to remove the acid residue. There were three main groups of pots, each with forty pots. One tomato transplant was placed in each pot for each group. Each of the three main pot groups was divided into two sub-main groups, one for control and one for water-limited stress (WL). Pots were arranged in a greenhouse and the Hoagland and Arnon, [26] nutritive solution (pH 5.9) was used twice a week to supply all of the pots. The greenhouse's conditions were 24±5 °C during the day (12 hours) and 17±3 °C at night (12 hours), with an average humidity of 61.4 - 65.6%. The greenhouse's sunlight availability, with an average of 12 hours of radiation, was maintained uniformly. Fifteen days post-transplanting, plants were placed in one of the three main sets' sub-main pot groups (SD-H_2O, 7.5% MEEst, and 15.0% MEEst treatments) exposed to WL (60% of soil relative water content, or SRWC) until harvest. For this study, a 60% SRWC watering schedule was suggested since it significantly reduced tomato growth without causing plant death (Table 2). Full irrigation volume (F-IV; 100% SRWC) was applied to the other sub-main pot group of the three main sets of foliar spraying treatments. The six treatments were therefore as follows: (1) SD-H_2O and irrigation at 100% SRWC; (2) 7.5% MEEst and irrigation at 100% SRWC; (3) 15.0% MEEst and irrigation at 100% SRWC; (4) SD-H_2O and irrigation at 60% SRWC; (5) 7.5% MEEst and irrigation at 60% SRWC; and (6) 15.0% MEEst and irrigation at 60% SRWC. Throughout the trials, all F-IV and WL treatments took into account the equality of nutrient concentrations in the nutritive solution. Weighing the pot every day to make up for the water loss helped regulate the required level of soil moisture based on the F-IV and WL treatments.

The trial treatments were arranged in a completely randomized factorial design. The pots were rotated after weighing daily to prevent systematic errors caused by eco-fluctuations.

2.3. Calculating soil relative water content (SRWC)

To calculate SRWC, the gravimetric method was applied to the F-IV and WL treatments (100 and 60% of SRWC). Daily, the amount of evapotranspiration from the pot water was compensated by weighing the pot, and the amount of water lost was added to the corresponding target SRWC [25], as follows:

$$SRWC\ (\%) = \left[\frac{(full\ pot\ weight - empty\ pot\ weight - dry\ soil\ weight)}{(full\ pot\ weight\ at\ field\ capacity - empty\ pot\ weight - dry\ soil\ weight)}\right] \times 100$$

2.4. Preparation of MEEst (maize grain embryos-derived natural extract enriched with some growth stimulators)

MEE was prepared according to Alharby et al. [11, 20] abd Alzahrani and Rady, [21] and. The concentrations of antioxidants, polyamines, phytohormones, osmosis-related compounds, and nutritional elements were measured in the resultant MEE. Nevertheless, it was found that MEE is lacking in a few essential elements, including selenium (Se), ascorbate (AsA), and gibberellic acid (GA_3). As a result, the MEE was enriched with these elements at concentrations of 1.5 mg, 20 mM, and 13 mg per L MEE, respectively. Consequently, MEEst was obtained for use in the tomato treatments in this study (Table 3).

The extracts were either used immediately or kept in the refrigerator (−20°C) until use.

2.5. Sampling

Nine plants were randomly selected from each treatment, and from each plant, the top third leaf was collected for analyses of the antioxidant system and physio-biochemical parameters.

2.6. Estimation of phytohormone contents

The auxin (IAA), gibberellic acid (GA_3), and cytokinins (CKs) profiling were evaluated by applying the gas chromatography–mass spectrometry (GC-MS) system, phytohormones were analyzed using a Clarus 680 GC with SQ8-T Mass Spectrometer system (Perkin Elmer, Waltham, MA, USA) fitted with an Elite-5MS capillary column (low bleed, 30 m × 0.25 mm × 0.025 μm film thickness; Perkin Elmer, Waltham, MA, USA), [14,27] . A 100mg fresh leafy sample was extracted in a 2 mL ice-cold solution containing 80 CH_3OH: 19.9 H_2O: 0.1 6N HCl (v/v/v). After cold centrifugation (25,000 r.p.m., 5 min), the supernatant collected was stored under −80 °C until use. The high-performance liquid chromatography (HPLC) system, (Shimadzu, C-R4A Chromatopac; SCL-6B systemcontroller) with a UV detector and C18column (39 mm × 300 mm), was used to evaluate the ABA level after extraction [27].

2.7. Evaluation of oxidative stress indices

The levels of malondialdehyde expressing lipid peroxidation, H_2O_2, and $O_2^{\bullet -}$ were evaluated following the Kubiś, [28], Velikova et al. [29], and Madhava Rao and Sresty, [30] methods, respectively.

2.8. Estimation of osmosis-related compounds and low molecular mass antioxidant compound contents

The anthrone method, Irigoyen et al. [31] was utilized to determine the total soluble sugar content (mg g^{-1} FW). Glycine betaine (GB) content was measured at 230 nm using a spectrophotometer following the procedure of Subbarao et al. [32], and the results were quantified using GB standard curves. Soluble protein content was assessed using Bradford's, [33] method. The evaluation of free proline (mg g^{-1} FW) was conducted using the acid-ninhydrin solution method, as described by Bates et al. [34]. The comprehensive protocols described by Kampfenkel et al. [35] and Griffth, 1980 [36] were used to assess the contents (μg g^{-1} FW) of glutathione (GSH) and ascorbate (AsA), respectively. HPLC techniques are employed by Nagy et al. [37] to ascertain the α-tocopherol content (μg g^{-1} FW).

2.9. Evaluation of antioxidant enzyme activities

For the enzyme activity assay, a 500.0 mg leaf sample was extracted using the Mukherjee and Choudhuri, [38] extraction method. The collected supernatant after homogenizing the sample and centrifugation (15, 000 × g, 10 min) of the homogenate was exploited. Superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), and ascorbate peroxidase (APX) activities were evaluated by exploiting the methods of Giannopolitis and Ries, [39], Smith et al. [40], Ali et al. [41], and Asada, [42].

2.10. Estimation of growth traits

Plant (45-days post transplantation) samples were taken from all treatments. Leaf numbers per plant were counted, and a LI-3000C leaf area meter (Portable, LICOR Inc., Lincoln, NE, USA) was exploited to measure plant leaf area. The plants were then divided into two parts, namely, shoots and roots. The fresh weight (FW) was recorded for them with an electric balance, and the lengths were measured with a 30 cm graduated ruler. After drying the plants at 70 ± 2°C, until constant weights were reached, the dry weight (DW) was taken.

2.11. Statistical analysis

The data obtained as the means of three trials conducted simultaneously were analyzed collectively using mixed models and tested for homogeneity of error variance [43]. The data were analyzed using the two-way ANOVA with the GLM procedure of Gen STAT (version 11) (VSN International Ltd., Oxford, UK). The LSD test was utilized to examine the differences among the means [44].

3. Results

Table 1 shows the optimal concentrations of MEEst, a natural extract derived from maize grain embryos enriched with gibberellic acid (GA_3), ascorbate (AsA), and selenium (Se), determined by a preliminary trial. It was found that MEEst at concentrations of 7.5 and 15.0% produced the best results for tomato growth exceeding 22.5% concentration. Additionally, all concentrations were exceeded by 7.5 and 15.0% MEEst; 7.5–30% of unenriched extract (MEE). Thus, in the primary trial, 7.5 and 15.0% MEEst were used. Furthermore, Table 2 shows that, in contrast to full irrigation volume (F-IV; 100% of soil relative water content; SRWC), the WL that significantly reduced tomato plant growth without causing plant death was 60% SRWC as the stress of water-limited (WL), whereas 40% SRWC did so. Thus, in the primary trial, 60% SRWC was applied as WL. Subsequently, a primary investigation was conducted in triplicate at the same time with two distinct water regimes: 60% SRWC as WL and 100% SRWC as F-IV. Additionally, tomato transplants should be sprayed with two MEEst concentrations: 7.5 and 15.0%. For the primary trials, a fully randomized factorial design was used. By thoroughly examining the outcomes of the primary trials shown in (Fig. 1-5), it was determined that: under F-IV and WL treatments, the 15.0% MEEst treatment significantly outperformed the 7.5% MEEst treatment in terms of improving hormonal contents, osmosis-related compound contents, and the activities of various antioxidant system components. Overall, all the results show that, when applied under WL, the 15.0% MEEst treatment is more effective than F-IV.

Table 1: A preliminary experiment to identify the best levels of maize grain embryos-derived natural extract (MEE) and the extract enriched with gibberellic acid, ascorbate, and selenium (MEEst) to apply to tomato (*Solanum lycopersicum* L. cultivar 023) in the main study.

Parameters	Unit	MEE or MEEst levels						
		07.5% MEE	15.0% MEE	22.5% MEE	30.0% MEE	07.5% MEEst	15.0% MEEst	22.5% MEEst
Total chlorophylls	(mg g^{-1} FW)	2.79±0.10e	3.14±0.11d	3.52±0.15c	3.50±0.14c	3.98±0.15b	5.08±0.18a	3.54±0.16c
Total carotenoids		0.69±0.01d	0.71±0.02d	0.78±0.02c	0.79±0.02c	0.86±0.02b	0.98±0.03a	1.01±0.04a
Free proline		0.67±0.02d	0.74±0.02c	0.75±0.03c	0.82±0.03b	0.82±0.03b	0.94±0.04a	0.93±0.04a
Total soluble sugars		1.33±0.04d	1.55±0.05c	1.68±0.06b	1.70±0.06b	1.72±0.06b	2.78±0.15a	2.69±0.14a
Leaf area plant^{-1}	(cm^{2})	27.9±0.81e	31.4±0.90d	32.1±0.95d	35.9±1.10c	39.7±1.08b	47.2±1.45a	35.7±1.04c
Number of leaves plant^{-1}		5.50±0.15e	6.04±0.20d	6.50±0.28c	6.54±0.29c	7.12±0.30b	8.29±0.33a	6.48±0.28c
Shoot length plant^{-1}	(cm)	17.5±0.42e	17.6±0.45e	18.4±0.60d	19.1±0.74c	19.9±0.87b	23.2±0.99a	19.8±0.80b
Root length plant^{-1}		11.0±0.20d	11.1±0.20d	11.6±0.28c	11.6±0.30c	12.2±0.49b	13.9±0.57a	11.7±0.33c
Shoot FW plant^{-1}	(g)	2.07±0.05e	2.29±0.08d	2.58±0.12c	2.55±0.09c	2.85±0.12b	3.64±0.18a	2.60±0.10c
Shoot DW plant^{-1}		0.72±0.02d	0.78±0.02c	0.80±0.02c	0.81±0.02c	0.89±0.02b	1.21±0.04a	0.87±0.02b
Root FW plant^{-1}		0.42±0.01e	0.46±0.01d	0.49±0.01c	0.50±0.01c	0.53±0.02b	0.73±0.03a	0.50±0.02c
Root DW plant^{-1}		0.20±0.00d	0.20±0.00d	0.22±0.00c	0.22±0.00c	0.26±0.00b	0.34±0.01a	0.22±0.01c

Values are means ± SE (n = 9). Mean values in each column followed by a different lower-case letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$. FW; fresh weight, DW; dry weight.

3.1. Response of hormonal contents of tomato to SRWC and MEEst

There were variations between the two irrigation regimes according to the phytohormone analyses (IAA, GA_3, Zeatin-CK, CKs, and ABA) (Fig. 1). In comparison to non-stressed plants, WL-stressed plants recorded a reduction in IAA by 26%, GA_3 by 33%, Zeatin-CK by 40%, and CKs by 36%, as well as higher ABA by 84%. Regarding the application of MEEst at a concentration of 15.0%, treated *Solanum lycopersicum* L. plants displayed a 23% increase in IAA, a 23% increase in GA_3, a 22% increase in Zeatin-CK and CKs, and a 36% decrease in ABA content in comparison to untreated plants (F-IV). Similarly, compared to the corresponding stressed control, MEEst at 15.0% under WL conditions significantly increased IAA by 37%, GA_3 by 53%, Zeatin-CK by 69%, and CKs by 59%, while decreasing ABA by 47%.

Table 2: A preliminary experiment to identify the irrigation level affecting tomato plants (*Solanum lycopersicum* L. cultivar 023) without causing death for use in the main study.

Parameters	Unit	Irrigation level treatments			
		100%	80%	60%	40%
Total chlorophylls	(mg g^{-1} FW)	2.51±0.21a	2.20±0.10b	1.15±0.06c	The plants died just before sampling.
Total carotenoids		0.62±0.02a	0.55±0.02b	0.29±0.01c	
Free proline		0.50±0.02c	2.14±0.07b	3.94±0.11a	
Total soluble sugars		2.53±0.19c	3.51±0.10b	4.62±0.15a	
Leaf area plant^{-1}	(cm^{2})	379±7.6a	229±4.5b	107±2.1c	
Number of leaves plant^{-1}		17.0±0.58a	13.0±0.44b	8.3±0.23c	
Shoot length plant^{-1}	(cm)	32.5±0.92a	27.0±0.84b	17.6±0.47c	
Root length plant^{-1}		19.8±0.50a	14.7±0.44b	10.0±0.36c	
Shoot FW plant^{-1}	(g)	15.0±0.48a	13.2±0.41b	6.7±0.21c	
Shoot DW plant^{-1}		2.51±0.18a	2.00±0.15b	1.06±0.08c	
Root FW plant^{-1}		10.29±0.40a	7.19±0.22b	3.64±0.12c	
Root DW plant^{-1}		1.98±0.12a	1.08±0.07b	0.62±0.04c	

Values are means ± SE (n = 9). Mean values in each column followed by a different lower-case letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$. FW; fresh weight, DW; dry weight.

Table 3: The major component contents (on a fresh weight; FW basis) detected in maize grain embryos-derived natural extract (MEE) and the extract enriched with gibberellic acid, ascorbate, and selenium (MEEst)

Component	Unit	Values	
		MEE	MEEst
Total soluble sugars	(mg g⁻¹ FW)	26.2	25.9
Total free amino acids		44.7	44.5
Free proline	(µmol g⁻¹ FW)	31.6	31.8
Ascorbic acid (AsA)		5.26	24.6
Glutathione (GSH)		8.28	8.16
α-Tocopherol		4.82	4.90
Flavonoids	(µg g⁻¹ FW)	14.9	14.8
DPPH radical-scavenging activity	%	78.6	88.2
Putrescine (PUT)	(µmol g⁻¹ FW)	10.4	11.1
Spermidine (SPD)		16.7	16.8
Spermine (SPM)		18.9	18.5
Gibberellic acid (GA_3)	(µg g⁻¹ FW)	2.86	4.22
Cytokinins (CKs)		4.14	4.08
Zeatin-type-CK		2.21	2.18
Salicylic acid (SA)	(µmol g⁻¹ FW)	2.96	3.01
Selenium (Se)	(µg g⁻¹ FW)	3.38	16.4
Nitrogen (N)		4.40	4.46
Phosphorus (P)		1.18	1.27
Potassium (K)		4.26	4.22

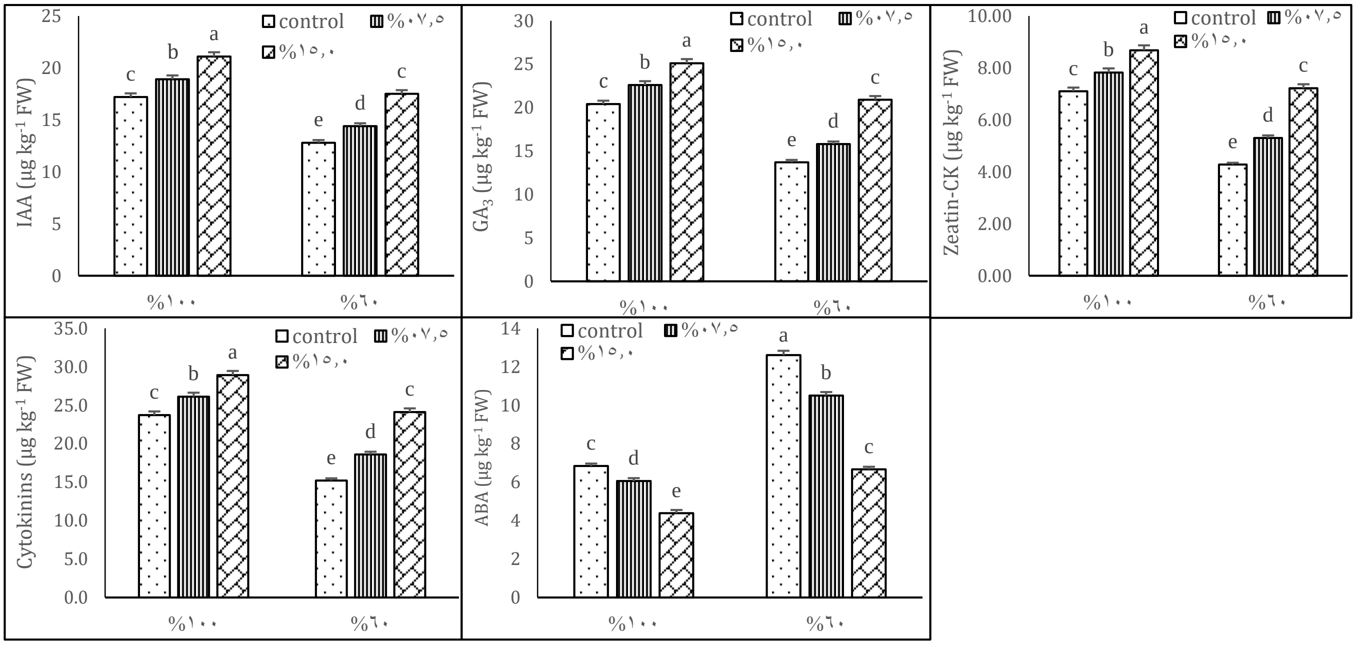

Fig.1. Effect of exogenous application of maize grain embryos-derived natural extracts (MEE) enriched with some growth stimulators (MEEst) on hormonal contents of tomato (*Solanum lycopersicum* L. cultivar 023) plants grown under two irrigation regimes (IR; 100 and 60% of soil water holding capacity). Values are means ± SE (n = 9). Mean values in each column followed by a different lower-case letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$. IAA; indole-3-acetic acid, GA_3; gibberellic acid, Zeatin-CK; Zeatin-type-cytokinin, CKs; cytokinins, and ABA; abscisic acid.

3.2. Response of oxidative stress indices of tomato to SRWC and MEEst

Lipid peroxidation, which is represented by the contents of superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA), was found to be useful among the oxidative damage indices found in this investigation (Fig. 2). When the irrigation level was reduced from IR100% to IR60%, the contents of MDA, H_2O_2, and $O_2^{\bullet-}$ increased by 60%, 98%, and 118%, respectively, compared to the full irrigated control. In reference to the MEEst applications, 15.0% MEEst considerably reduced MDA, H_2O_2, and $O_2^{\bullet-}$ levels by 5%, 24%, and 27%, compared to the control (F-IV). In comparison to the corresponding stressed control, 15.0% MEEst under WL conditions significantly reduced MDA, H_2O_2, and $O_2^{\bullet-}$ contents by 34%, 26%, and 59%, respectively.

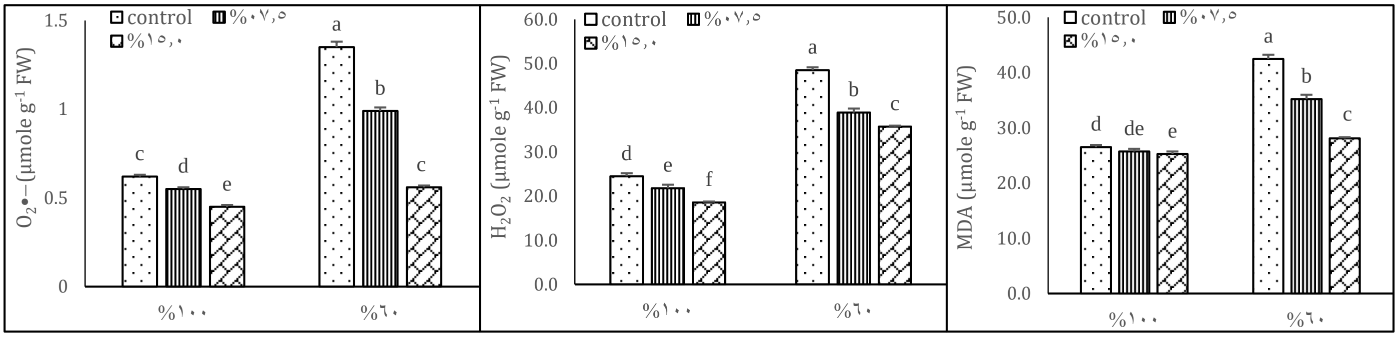

Fig.2. Effect of exogenous application of maize grain embryos-derived natural extracts (MEE) enriched with some growth stimulators (MEEst) on oxidative stress indices in tomato (*Solanum lycopersicum* L. cultivar 023) plants grown under two irrigation regimes (IR; 100 and 60% of soil water holding capacity). Values are means ± SE (n = 9). Mean values in each column followed by a different lower-case letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$. $O_2^{\bullet-}$; superoxide, H_2O_2; hydrogen peroxide, and MDA; malondialdehyde.

3.3. Response of osmosis-related compounds and low molecular mass antioxidant compound contents of tomato to SRWC and MEEst

Results presented in Fig. 3 show that compared with fully irrigated control, the contents of total soluble protein in WL-stressed tomato plants decreased by 60%, while osmosis-related compounds such as free proline, soluble sugars, and glycine betaine significantly increased by 120%, 482%, and 174%, respectively. Comparing MEEst 15.0% to the corresponding control, the contents of soluble sugars increased by 245%, glycine betaine by 63%, total soluble protein by 36%, and free proline by 53% under optimum irrigation (F-IV). In comparison to the corresponding control, the osmotically stressed plants treated with 15.0% MEEst showed increases in free proline, soluble sugars, glycine betaine, and total soluble protein contents of 51%, 43%, 23%, and 106%, respectively.

Comparing plants that received 60% irrigation to those that received well water, the contents of low molecular mass antioxidant compounds [ascorbic acid (AsA), glutathione (GSH), and α-tocopherol (α-TOC)] (Fig. 3) increased by 70%, 143%, and 145%, respectively. When applying 15.0% MEEst during full irrigation, the activities of AsA increased by 46%, GSH by 71%, and α.TOC by 100% when compared to the corresponding control. However, the activities did not reach the levels achieved during WL stress. In comparison to the corresponding control, treatment with 15.0% MEEst increased these non-enzymatic antioxidant activities under WL stress by 32%, 47%, and 48%, respectively. These improvements significantly outperformed those obtained under full irrigation (F-IV) treatment.

3.4. Response of enzyme activities of tomato to SRWC and MEEst

When compared to plants that were well-watered (F–IV), the contents of the enzymatic antioxidant activities [superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR)] (Fig. 4) increased by 100%, 135%, 85%, and 433%, respectively. This increase occurred under an irrigation level of 60%. When treated with 15.0% MEEst under full irrigation, the activities of SOD increased by 22%, APX by 96%, CAT by 31%, and GR by 133% when compared to the corresponding control; however, these increases did not reach the levels seen under WL. In comparison to the corresponding control, treatment with 15.0% MEEst significantly increased these enzymatic antioxidant activities under WL stress by 39%, 36%, 71%, and 69%, respectively. These results significantly outperformed those obtained under full irrigation treatment.

3.5. Response of growth traits of tomato to SRWC and MEEst

As illustrated in Fig. 5, the growth traits of *Solanum lycopersicum* L. plants (leaf area plant^{-1}, the number of leaves plant^{-1}, shoot length plant^{-1}, root length plant^{-1}, shoot fresh weight (FW) plant^{-1}, shoot dry weight (DW) plant^{-1}, Root FW plant^{-1}, and Root DW plant^{-1}) were significantly reduced by WL stress in comparison to the control group by 64%, 38%, 30%, 17%, 47%, 44%, and 55%, respectively. In contrast to the corresponding control, the application of 15.0% MEEst under full irrigation significantly increased all of the aforementioned growth traits by 118%, 13%, 33%, 25%, 24%, 104%, 69%, and 54%. In comparison to the corresponding stressed control, MEEst at 15.0% increased the aforementioned growth traits by 56%, 15%, 20%, 18%, 94%, 73%, 57%, and 56%, respectively, under deficit irrigation.

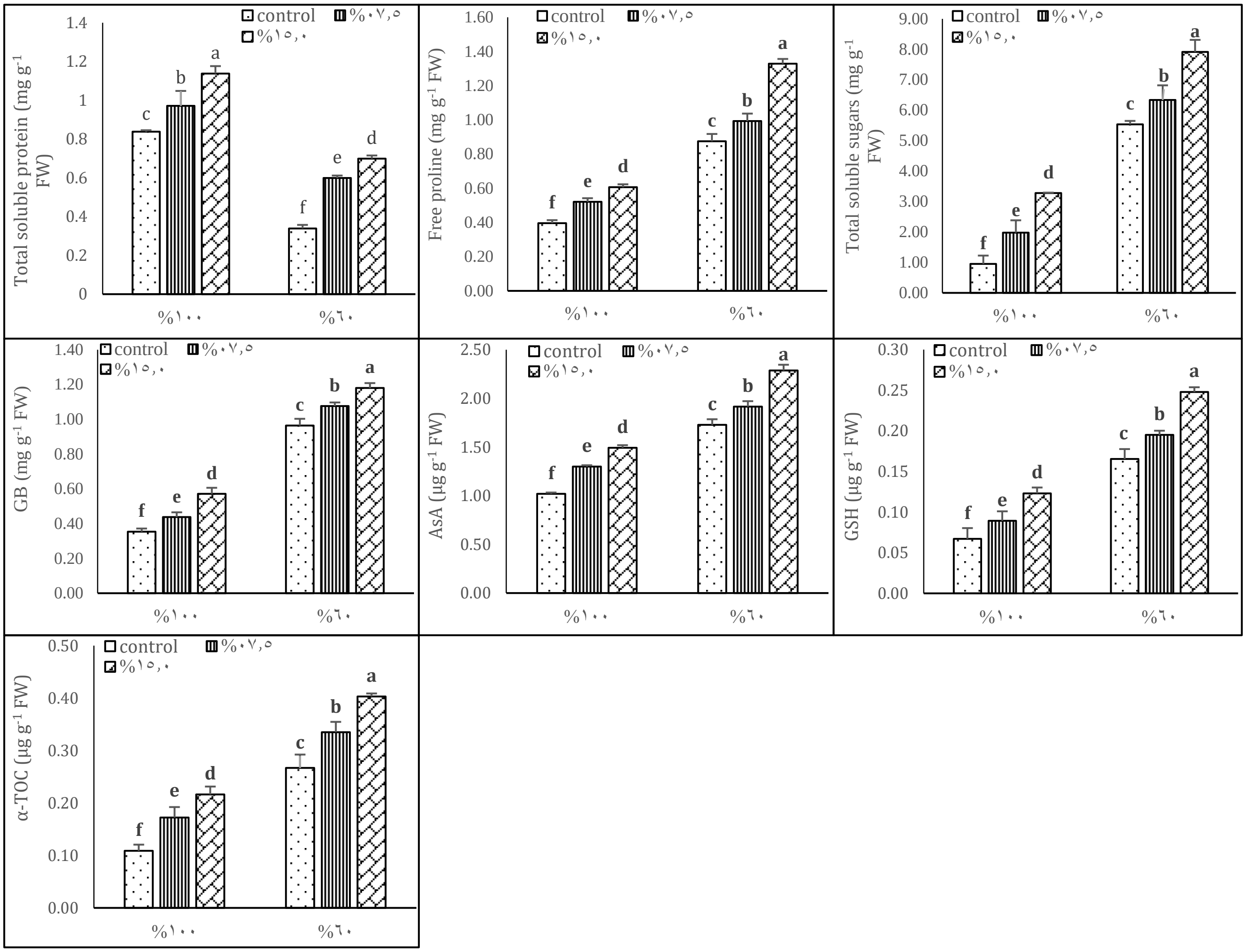

Fig.3. Effect of exogenous application of maize grain embryos-derived natural extracts (MEE) enriched with some growth stimulators (MEEst)on osmosis-related compounds and low molecular mass antioxidant compound contents of tomato (*Solanum lycopersicum* L. cultivar 023) plants grown under two irrigation regimes (IR; 100 and 60% of soil water holding capacity). Values are means ± SE (n = 9). Mean values in each column followed by a different lower-case letter are significantly different by Fisher's least-significant difference test (LSD) at *P* ≤ 0.05. GB; glycine betaine, AsA; ascorbic acid, GSH; glutathione, and α-TOC; α-tocopherol.

4. Discussion

Prior research indicates that the natural extract derived from maize grain embryos (MEE) is very beneficial in mitigating the adverse effects of plant stresses, such as the stress resulting from low water availability (WL) [11, 45]. Moreover, no studies on the application of MEE enriched with biostimulators like gibberellic acid (GA_3), ascorbate (AsA), and selenium (Se) (MEEst) to tomato transplants have been planned as of yet. In this study, tomato plants treated with 15.0% MEEst yielded significantly higher growth and productivity than those grown under WL, with growth and productivity gains exceeding 7.5% MEEst. Numerous research studies have clarified significant alterations in the morpho-physio-biochemical indices as well as various antioxidant defense system components (such as enzymes, low molecular mass antioxidant compounds, and compounds related to osmosis) of various plants grown under WL [25, 46]. In this study, positive changes were observed with the hormonal status (Fig. 1), levels of low molecular mass antioxidant compounds and osmosis-related compounds (Fig. 3), and enzyme activities (Fig. 4), resulting in satisfactory tomato growth under WL (Fig. 5). These changes were caused by treating tomato transplants with 15.0% MEEst.

Alharby et al. [25] reported that WL caused the closure of stomata, altered the typical tendency of components of the antioxidant defense system, and ultimately resulted in the plant's death. On the other hand, under WL, this study demonstrated the important advantages of spraying tomato transplants with MEEst. Based on the biostimulators found in MEEst (Table 3), biochemicals related to osmosis (proline, free amino acids, soluble sugars, etc.), low molecular mass antioxidants (glutathione, α-tocopherol, flavonoids, etc.), polyamines (spermine, spermidine, and putrescine), phytohormones (cytokinins, zeatin-type-cytokinin, salicylic acid, etc.), and nutrients (N, P, K, etc.) can be used to secure the high benefits of MEEst. besides the GA_3, AsA, and Se enrichments obtained for the MEEst examined in this work. The enriched MEEst was more effective in overcoming stress-induced damage to crop plants than the unenriched MEE (Table 3).

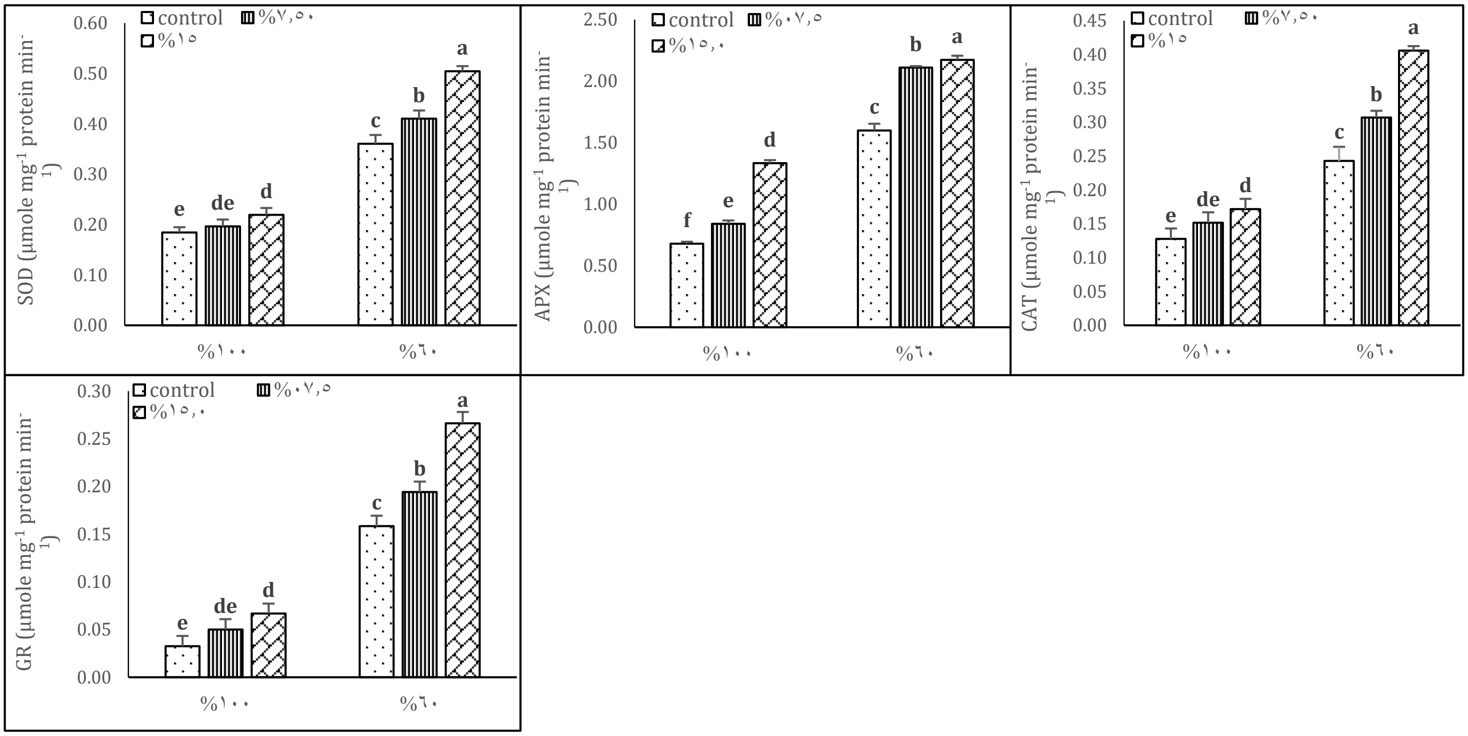

Fig.4. Effect of exogenous application of maize grain embryos-derived natural extracts (MEE) enriched with some growth stimulators (MEEst) on enzymatic activities of tomato (*Solanum lycopersicum* L. cultivar 023) plants grown under two irrigation regimes (IR; 100 and 60% of soil water holding capacity). Values are means ± SE (n = 9). Mean values in each column followed by a different lower-case letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$. SOD; superoxide dismutase, APX= ascorbate peroxidase, CAT; catalase, GR; glutathione reductase.

Tomato transplants may absorb MEEst biostimulators, such as GA_3, AsA, and Se (Table 3), during transplants spraying. This enrichment and acceleration of metabolic processes will give the transplants strong growth resistance to WL. This report examines how MEEst can maintain pigments related to photosynthesis under WL, and how this has a positive impact on growth (Fig. 5). Accordingly, Alharby et al. [25] and Alghamdi et al. [47] reported a positive correlation between high growth and photosynthesis effectiveness in tomato plants, which they attributed to the maintenance of PSII function, activation of the antioxidant system, and hormonal balance (Figs. 1, 4, and 5). This can be attributed to the beneficial effects of MEEst biostimulators (Table 3), which incentivized tomato plants to flourish under WL circumstances. These encouraging outcomes could be linked to MEEst's ability to counteract the harmful effects of WL. MEEst offered effective mechanisms to do this, such as the upregulation of compounds related to osmosis, antioxidants with low molecular mass, and enzymes (Figs. 3 and 4) that remove excess reactive oxygen species ($O_2^{\bullet-}$ and H_2O_2) and subsequently lower malondialdehyde (MDA) (Fig. 2). The ability of nutrients to maintain the intercellular ion balance necessary for the biosynthesis of additional osmosis-related compounds and to contribute to the ideal water content for a healthy plant metabolism is one of MEEst's effective mechanisms.

According to Ullah et al. [48], phytohormones are crucial in various physiological, biochemical, and molecular processes in plants that help to reduce WL stress, which was considerably reduced while abscisic acid (ABA) content increased (Fig. 1). In well-watered and stressed plants, treatment with MEEst increased levels of gibberellic acid, indole-3-acetic acid, Zeatin-type-cytokinin, and cytokinins while ABA content decreased (Fig. 1). The MEEst is rich in several classes of phytohormones, especially IAA, GA_3, and cytokinins (Table 3), and has enhanced cell metabolism, which may account for this increase in hormonal concentration [45]. Plant hormone content rises as a result of this enhanced cell metabolism. By either stimulating shoot growth or regulating processes to prevent plant growth, phytohormones reduce the effects of stressors and increase rates of survival [25]. This allows them to control plant growth activities [49,50] . According to our research, higher levels of MEEst (Fig. 1) hormone content was linked to increased antioxidative defense system activity (Fig. 3 and 4) as well as suppression of ROS, $O_2^{\bullet}$ and H_2O_2 levels. These factors reduced MDA levels (Fig. 2) and enhanced photosynthesis efficiency and plant performance. Furthermore, under stress, ABA is typically antagonistic to GA_3 and cytokinins [21, 51].

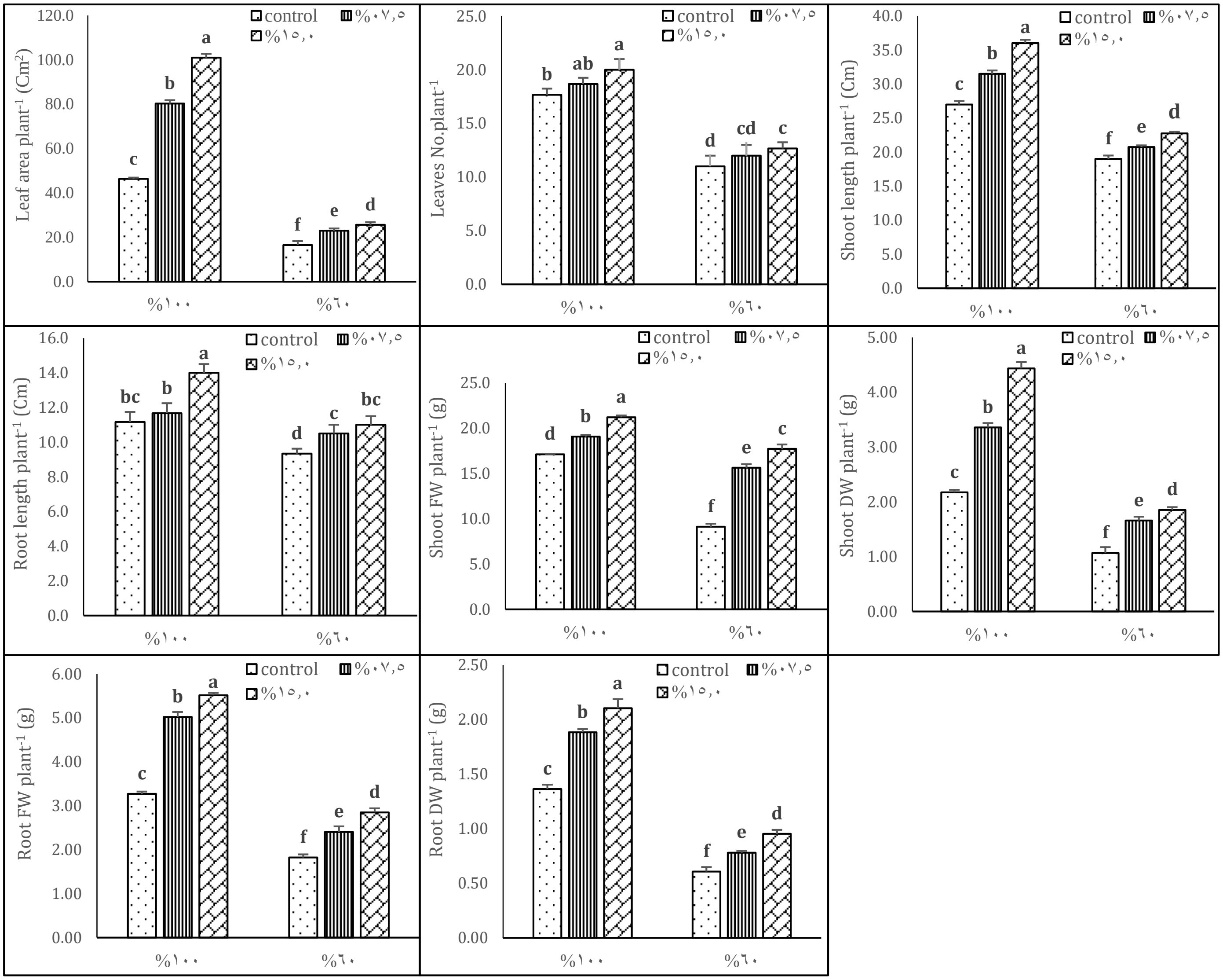

Fig. 5. Effect of exogenous application of maize grain embryos-derived natural extracts (MEE) enriched with some growth stimulators (MEEst) on growth traits of tomato (*Solanum lycopersicum* L. cultivar 023) plants grown under two irrigation regimes (IR; 100 and 60% of soil water holding capacity). Values are means ± SE (n = 9). Mean values in each column followed by a different lower-case letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

Under typical environmental circumstances, plants' metabolism of ROS maintains a dynamic equilibrium between continuous production and elimination, making membrane lipid peroxidation difficult to occur. However, as our study revealed, it is widely known that WL disrupts cellular homeostasis, which results in an excessive cellular build-up of ROS like hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet}$) (Fig. 2), which oxidatively damages various plant cell components [52], possibly inflicting damage to the plasma membrane and even resulting in cell death.

These effects may account for the observed excessive rise in H_2O_2 on the one hand and the increased rate of lipid peroxidation as demonstrated by MDA on the other in our investigation. MDA is a stable metabolite of peroxidation, and its content can indicate how much oxygen free radicals have damaged plant cells [53, 54]. A higher MDA concentration (Fig. 2) suggested that under WL, plants' antioxidant enzyme system was disrupted, due to increasing ROS production and increased membrane lipid peroxidation, which ultimately resulted in membrane damage.

The impact of MEEst treatment on MDA accumulation was examined as an indicator of oxidative damage to cellular membranes caused by excessive ROS production. Reduced levels of H_2O_2 and $O_2^{\bullet}$, and consequently reduced lipid peroxidation (MDA) and membrane leakage (EL), were obtained with the MEEst-treated plants under normal condition or WL, as previously obtained in some works Rehman et al. [17], Rady et al. [45] (Fig. 2). The MEEst-mediated enhanced membrane integrity (due to reduced membrane EL and MDA) could be attributed to the bioactive components in present MEEst (Table 3), which contributed to the maintenance of the antioxidant system's components, in addition to the low peroxidation levels, which were considerably influenced by WL. It contributed effectively to accumulating osmosis-related compounds (Fig. 3) to protect cells by keeping a balance between the osmotic strength of cytosol and osmotic strength of cellular vacuole and that of the external environment [11,55]. Furthermore, it promoted a high accumulation of α-tocopherol (α-TOC) (Fig. 3), which is important for preserving cell turgor and membrane integrity [56].

Plants use the accumulation of various organic substances and compounds related to osmosis to protect their leaf tissue from dehydration. Under WL conditions, these molecules are essential for osmotic adjustment and water uptake [57]. This study examined the accumulation of proline, soluble sugars, and glycine betaine in the leaves. Our findings showed that, compared to the plants that received regular irrigation, WL increased the levels of compounds related to osmosis (Fig. 3). These findings concurred with some earlier studies Ibrahim, [58], Ibrahim et al. [59]. In addition to acting as osmolytes, they also serve as hydrophobic protectants and/or N storage compounds for enzymes and cellular structures [60].

The induction of a burst of compatible osmosis-related compounds, such as free proline, total soluble sugars, and glycine betaine in well-watered and WL-affected plants treated with MEEst, provides additional evidence that MEEst application improves plant WL tolerance (Fig. 3). These osmosis-related substances maintain cell turgor pressure for healthy metabolism in addition to regulating endogenous osmotic pressure to shield cell membranes from WL damage. According to Semida and Rady, [51], they can either directly or indirectly affect the expression of genes related to metabolic processes like photosynthesis, defense, and storage functions. The compounds related to osmosis may accumulate because of their increased biosynthesis, which is catalyzed by MEEst plants, or because they are absorbed from MEEst. Proline, soluble sugars, and glycine betaine accumulations improved as a result of MEEst treatment, which helped plants under WL stress maintain water balance and prevent elevated EL and MDA levels in plant tissues for protection of protein turnover, enzymatic activities, and healthy metabolic processes. These findings point to MEEst's defense of healthy metabolic pathways and efficient osmoregulation. Similar results were obtained by Alharby et al. [20] and Ahanger and Agarwal, [61].

Ascorbic acid (AsA), glutathione (GSH), and α-TOC are examples of non-enzymatic low molecular weight antioxidants that are known to regulate the level of ROS in plant tissues [62, 63]. The primary components of the AsA-GSH cycle, AsA and GSH, are thought to be an efficient defense mechanism that allows tomato plants to grow and/or adopt a complex antioxidant defense system capable of mitigating and recovering from oxidative damages generated by excessive ROS [64,65]. Ascorbate can donate electrons to a variety of enzymatic and non-enzymatic reactions, making it one of the most potent ROS scavengers. By directly scavenging $O_2^{\bullet -}$ and OH^- and by regenerating α-tocopherol from tocopheroxyl radical, it can give protection to membranes. In chloroplast, ascorbate acts as a cofactor of violaxanthin de-epoxidase, thus sustaining dissipation of excess excitation energy [66, 67]. Ascorbate is crucial for maintaining the activities of enzymes that contain prosthetic transition metal ions in addition to its role in the ascorbate-glutathione cycle [68]. The ascorbate redox system consists of L-AsA, mono-dehydroascorbate and dehydroascorbate. Both oxidized forms can be chemically reduced by glutathione to ascorbate [69]. To preserve cell membranes and their functions, α-TOC can effectively scavenge a variety of ROS and drastically lower the amounts of MDA and H_2O_2.

Low H_2O_2 and $O_2^{\bullet -}$ levels under MEEst treatment were associated with higher GSH and AsA content in both WL-stressed and well-watered plants (Figs. 2 and 3). This emphasizes MEEst and GSH-AsA's roles in maintaining the balance of ROS. Our findings showed that in both WL-stressed and well-watered plants, MEEst dramatically raised the concentrations of AsA, GSH, and α-TOC (Fig. 3). This could be because MEEst, a plant biostimulant, contains a lot of growth stimulants. It has notable amounts of AsA and GSH in addition to abundant amounts of soluble sugars, free proline, different mineral nutrients, phytohormones, gibberellic acid, indoles, and zeatin.

To maintain ROS levels and shield plants from oxidative stress, plants have sophisticated antioxidant systems that work well together (70). In addition to being important enzymes in the ascorbate-glutathione cycle, superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR) are premium biochemical indicators of stress. They are mainly responsible for the detoxification of ROS and defense responses against different stresses [57]. Increasing the activities of these enzymes in this study under WL and well-watered conditions may indicate how well they cooperate to detoxify ROS from the cells. Numerous researchers have examined the relationship between WL tolerance in plants and the activity of the antioxidant enzyme system, but the findings have been mixed and inconsistent [71,72].

Tomato plants are shielded from oxidative stress (H_2O_2 and $O_2^{\bullet -}$) induced by WL by the MEEst treatment, which significantly increased the activity of antioxidant enzymes (fig. 4) and increased the contents of AsA, GSH, and α-TOC (Fig. 4). Under various stresses, comparable outcomes have been obtained in the Semida and Rady, [51], Rady et al. [45]. Identical outcomes were also attained in well-watered environments. Integrally, after dismutating $O_2^{\bullet -}$ to H_2O_2 by SOD, APX and CAT convert H_2O_2 to H_2O and O_2 and subsequently damage to membranes decreased (Fig. 2), improving WL tolerance in these plants. According to [63], this mechanism lessens the production of hydroxyl radicals. Since MEEst is a rich source of bioactive stimulants, it may stimulate SOD up-regulation to further dismutate $O_2^{\bullet -}$ to H_2O_2. GR is essential for maintaining AsA homeostasis in chloroplasts and is involved in the conversion of GSSG to GSH [70].

To improve tolerance strategies against any potential damage from oxidative stress in tomato plants, MEEst can stimulate and upregulate the AsA-GSH cycle, which is the ROS-scavenging pathway that includes all antioxidants (AsA, GSH, α-TOC, SOD, APX, CAT, and GR) (Figs 3 and 4). The WL-stressed tomato plants that were pretreated with MEEst showed a significant reduction in ROS accumulation via the AsA-GSH cycle as a consequence of this strong improvement in the antioxidant system. This increased protection of the photosynthesis pathways led to improved tomato plant performance. NADPH, GSH, and AsA all participate actively in a series of redox reactions known as the AsA-GSH cycle [67]. Through this cycle, APX and CAT scavenge H_2O_2 molecules in the cell cytosol and chloroplasts, preventing H_2O_2 diffusion to other organelles and averting damage. The enhanced performance of the AsA–GSH cycle pathway as a result of MEEst pretreatment successfully preserved redox components, such as AsA and GSH. The effects of oxidative stress induced by WL were mitigated by these redox components. In this study, tomato plants' heightened resistance to WL stress coincided with elevated activity of the antioxidant system. Alharby et al. [20] and Ahanger et al. [73] obtained similar results.

Our findings from this investigation showed that, in contrast to plants that received an appropriate water supply, WL inhibited plant growth and total chlorophyll. WL has been shown to cause stomatal closure [74], raise lipid peroxidation (measured as MDA), and release an excessive amount of ROS (Fig. 2) [52]. which ultimately results in the direct inhibition of chlorophyll degradation and photosynthesis [57]. Due to abnormalities in metabolic processes and an increased rate of respiration brought on by the increased energy requirements, meristem and cell expansion activities were reduced in tomatoes, limiting their growth [75].

The internal antioxidant systems of tomato plants were strengthened by MEEst treatment to withstand these undesired results of WL stress and maintain plant life. The increased content of photosynthetic pigments, enhanced compatible solute accumulation, improved cell membrane stability, induction of enzymatic and non-enzymatic antioxidant defense machinery, and the richness of MEEst—especially after its enrichment with (AsA, GA_3, and Se) in phytohormones, auxins, cytokinins including zeatins and gibberellins—all contribute to the improved growth and production of these well-watered and stressed plants (Table 3). Therefore, one possible method for increasing plants' ability to withstand WL stress is by inducing hormonal homeostasis with MEEst. This hormonal homeostasis mechanism may operate as an anti-stress network supporting the plant's response to WL stress through intricate interactions between auxins, cytokinins, and gibberellins, as well as between plant hormones and other biostimulants. Furthermore, the MEEst bioactive components are necessary to promote plant development and growth, including apical meristem cellular enlargement and division in response to various stresses [17, 51]. Therefore, it is important to note, that MEEst is crucial for improving plant physiology during WL and thereby causing a healthy metabolic state, which in turn promotes healthy plant growth and development (Fig. 5). Yan et al. [76] showed a relationship between the plant's biomass (growth) and its water content .

Stressed and well-watered tomato plants in the current study grew more when MEEst was applied (Fig. 5). This is because the MEEst treatment produced a large number of catalysts in the plants. By boosting photosynthetic efficiency, stomatal opening, osmoregulation, membrane stability, assimilate transport from source to sink, enzyme activity, and carbohydrate synthesis, MEEst plays a critical role in plant growth and productivity. Furthermore, better nutrient absorption might be the cause of these favorable results [17, 45].

5. Conclusions

Our study's findings suggest that a useful tactic would be misting water-stressed (WL) tomato plants with MEEst, a naturally occurring extract from maize grain embryos that is enriched with selenium (Se), ascorbate (AsA), and gibberellic acid (GA_3). This tactic can effectively encourage biomass accumulation and plant growth. The application of MEEst by foliar spraying tomato plants was crucial in promoting growth, non-enzymatic antioxidants, hormonal content, and enzymatic antioxidants. This led to the inhibition of lipid peroxidation and oxidative damage caused by reactive oxygen species ($O_2^{\bullet-}$ and H_2O_2). In our study, the antioxidant and hormonal components of MEEst worked as a natural biostimulant, interacting to benefit tomato plants under WL stress. To enhance WL stress tolerance in tomato plants, the results of this study suggest using MEEst as an efficient novel biostimulator for tomatoes. This will help to promote various physiological and metabolic processes.

Author Contributions

Conceptualization,M. M. Rady; Methodology, Kh. A. Hemida; Validation, M. M. Rady and H. M. Abbas; Formal analysis, S. A. Abdel-Hameed; Investigation, Kh. A. Hemida and S. A. Abdel-Hameed Data curation, M. M. Rady and H. M. Abbas; Writing—original draft preparation, Kh. A. Hemida and S. A. Abdel-Hameed; Writing—review and editing, Kh. A. Hemida and S. A. Abdel-Hameed; Visualization, H. M. Abbas and R. K. Kamel; Supervision, M. M. Rady and Kh. A. Hemida. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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