



Exogenous maize grain embryonic extract enriched with some biostimulants confers drought stress tolerance by promoting tomato's growth, photosynthetic efficiency, antioxidant defense, and yield and quality

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ABSTRACT

One of the primary stresses affecting tomato plant growth and yield is water limitation (WL). Using a completely randomized design with three replications, the effects of WL alone or with exogenous foliar application of maize grain embryos extract enriched with bio-stimulant (e.g., gibberellic acid, ascorbate, and selenium) (MEEst) on tomato plant growth and some related parameters. WL decreased leaf pigment levels, photosynthetic efficiency, water use efficiency (WUE), nutrient content, relative water content, and membrane stability index; however, increased electrolyte leakage. These negative effects resulted in a clear decrease in the growth parameters and yield. However, by applying MEEst as foliar spraying at two concentrations (i.e., 7.5 and 15.0%) to the tested plants growing under two irrigation regimes (100 and 60% of soil water holding capacity), the detrimental effects of WL stress were lessened. It also improved photosynthesis and leaf pigmentation, decreased electrolyte leakage, and raised membrane stability index, nutrient content, relative water content, and WUE. Positive effects are observed in the growth parameters, yield, and fruit quality. Thus, MEEst is a useful, long-lasting, and eco-friendly approach for reprogramming plant responses and mitigating the deleterious consequences of WL stress.

1. Introduction

Tomatoes are still the most extensively grown and eaten vegetable [1]. All economic sectors, regardless of developed or developing countries, view it as a staple food crop that benefits a diverse range of people. The final importance of the vegetable crop is demonstrated by nearly 4.2 million hectare (ha) that produce approximately 100 million tons of tomatoes annually. Flavonoids, lutein, beta-carotene, lycopene, and vitamins C and E are among the components of tomatoes that are good for human health. Macro- and micronutrient contents such as potassium (K), phosphorous (P), magnesium (Mg), calcium (Ca), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) [2].

Climate change is putting agricultural production worldwide in jeopardy. According to Farooq et al. [3], the three primary markers of climate change are rising average temperatures, changing precipitation patterns, and an increase in the unpredictability of extreme events. This led to significant losses in the agricultural sector. A net estimated loss of \$50 billion in agricultural production could arise from water limitation (WL) [4]. Future temperature extremes and more frequent summer droughts have been predicted by modelling studies [5, 3]. WL stress, which dramatically lowers crop plant productivity globally, is one of the most harmful environmental stresses brought on by climate change [6]. Reactive oxygen species (ROS) build up because of a variety of physiological alterations and metabolic process impairments brought on by it [7]. Disruption of photosynthetic pigment synthesis and cell membrane permeability are additional effects of WL stress. These negative consequences have an adverse effect on plant growth, productivity, and yield quality [8, 9].

However, plants use intricate defense mechanisms, such as the antioxidant defence system, to withstand WL stress [6]. The protective antioxidant system consists of two primary components: non-enzymatic antioxidants such as glutathione, ascorbate, and α -tocopherol, and enzymatic antioxidants such as glutathione peroxidase, superoxide dismutase, and ascorbate peroxidase [10].

Most of the time, the elements of a plant's endogenous antioxidant system prevent them from withstanding severe stress. Thus, it is advised to increase plant's resistance to WL stress by using exogenous applications (such as antioxidants and plant extracts) [11]. Because of the high ROS

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production that prevents the antioxidant components from scavenging, prolonged exposure to WL stress results in significant damage and eventually kills plant cells [12]. Thus, it is crucial to develop some of the methods for helping plants grow under stress [13,14].

Plant extracts are rich sources of bioactive stimuli and using them is one of the most important strategies according to several studies [15, 16]. Plant extracts, because of the biostimulants they contain, are an interesting environmentally friendly invention that improve resistance to a range of abiotic stressors and flowering, plant growth, fruit development, crop yield, and nutrient utilization efficiency [17, 18]. The richness of maize grain embryos extract (MEE) in cytokinins (CKs), especially zeatin-type cytokinin (Z-CK), auxins, especially indole-3-acetic acid, gibberellins, antioxidants, and essential nutrients to enhance morpho-physio-biochemical attributes for catalyze plant tolerance against unfavorable conditions makes MEE an organic biostimulant [16].

In the plant kingdom, selenium (Se) is a well-known beneficial element that is known to carry out a variety of advantageous functions in plants [19]. In a range of crop plants, exogenous Se application exhibits considerable promise for improving plant growth and development [20]. Research has shown that low concentrations of selenium (Se) improve water status, photosynthetic efficiency, and membrane integrity. They also improve the uptake and assimilation of essential nutrients, as well as the assimilation of N and carbohydrates. Additionally, they reduce the production of ROS- and lipid peroxidation. Growth, biomass, and leaf area are all increased by low concentrations of Se. Gibberellic acid (GA₃) is a crucial signaling molecule that controls a wide range of physiological and biochemical processes, such as the development of fruits, the induction of flowers, vegetative growth, and seed germination [21]. Many studies have examined the potent role that GA₃ plays in enhancing resistance to abiotic stressors like WL [22- 25]. Ascorbic acid (AsA) functions as a reductant and is crucial for protecting plant tissues from harmful oxidative damage [26, 27]. It has an important role in organogenesis, such as cell division, differentiation, and senescence [27]. It also helps to protect proteins and lipids and enhances tolerance against various abiotic stresses, such as WL [28]. Thus, AsA has been demonstrated to regulate photosynthesis, transpiration, and plant growth [29].

This study aimed to investigate the potential benefits of treating plants with a natural extract derived from maize grain embryos-enriched with gibberellic acid, ascorbate, and selenium (MEEst) to enhance plant growth and productivity by mitigating the adverse effects of drought. It accomplishes this by favorably influencing the build-up of organic solutes, ionic homeostasis, defense mechanisms of enzymatic and non-enzymatic antioxidants, and plant hormonal balance. The study also investigated the possible advantages of employing MEEst as exogenous plant growth enhancer. This strategy may provide a new avenue for preventing drought mediated damage, and further studies are required to unravel the underlying mechanisms.

2. Materials and Methods

2.1. Tomato transplant source and preparation for planting

The tomato (*Solanum lycopersicum* L. cultivar 023) transplants were obtained from the Egyptian Ministry of Agriculture's nurseries. Following a health and uniformity inspection, the tomato transplants that was both ideal for root development were transplanted in the soil. Following the transplanting, an application of MEEst foliar spraying was made, and the transplants were divided into three groups, each comprising forty transplants. Sterile deionized water (SD-H₂O) was sprayed foliarly on transplants in the first main group (control for the other 2 main groups). Transplants were foliar sprayed with MEEst at concentrations of 7.5 and 15.0% for the second and third main groups, respectively. Three times, 15, 30, and 45 days after transplanting, the tomato plants were sprayed. To ensure the best penetration, a few drops of Tween-20 were added to the spraying solution to act as a surfactant. Based on tomato growth outcomes 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

One hundred and twenty black plastic pots (30 cm diameter and 30 cm depth) were proposed for this investigation. Following decontamination, 9.5 kg of a medium made up of 90.0% pure sand, 6.5% compost, 3.0% vermicompost, and 0.5% humic acid was added to each pot [30]. Prior to this, commercial acid was used to thoroughly clean the sand of all ions, and SD-H₂O was used to remove the acid residue. There were three main groups of pots, each with forty pots. One tomato transplant was transplanted in each pot for each group. Each of the three main pot groups was divided into two sub-main groups, one for regular irrigation and one for the stress of WL, after the pots in each group had been arranged in a greenhouse. The Hoagland and Arnon, [31] nutritive solution (pH 5.9) was used twice a week to supply all the pots. After 15 days of transplantation, plants were placed in one of the three main set's sub-main pot groups (SD-H₂O, 7.5% MEEst, and 15.0% MEEst treatments) and exposed to WL (60% of soil relative water content; 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 spray treatments. The six treatments were therefore as follows: There are six different ways to apply foliar spraying to transplants: (1) using SD-H₂O and irrigation at 100% SRWC; (2) using 7.5% MEEst and irrigation at 100% SRWC; (3) using 15.0% MEEst and irrigation at 100% SRWC; (4) using SD-H₂O and irrigation at 60% SRWC; (5) using 7.5% MEEst and irrigation at 60% SRWC; and (6) using 15.0% MEEst and irrigation at 60% SRWC. Throughout the trials, F-IV and WL (i.e., 60% of SRWC) treatments were conducted with consideration for the equality of nutrient concentration in the nutritive solution. Weighing the pot every day to make up for the water lost helped regulate the required level of soil moisture based on the F-IV and WL treatments. The trial treatments were arranged in a 2 (irrigation levels) × 3 (MEEst concentrations) factorial design in twenty randomized replicates (i.e., pots). The pots were rotated after weighing daily to prevent systematic errors caused by eco-fluctuations.

2.3. Calculating soil relative water content (SRWC)

The F-IV and WL treatments (100 and 60% of SRWC) were subjected to the gravimetric method to calculate SRWC. Every day, the pot's weight was used to calculate the amount of evapotranspiration it produced, and the amount of water lost was added to the matching target SRWC in the manner described below:

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

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

Using the genotype of native maize grown in Egypt, the techniques described in Alzahrani and Rady [32] and Alharby et al. [34] were used to optimize the examined extracts from maize grain embryos. In the laboratory, a spotless bench was covered with a fresh local cloth. On the cloth, a thin layer of dampened cotton, about 2 cm thick, was applied. Then, half of the germination medium (cloth + moistened cotton) was covered with a layer of maize grains, about 2 cm thick. The remaining half was then applied to the grains, which were then left until they had fully germinated and had radical lengths of 1/2 cm. Following their extraction from the grains, the germinated embryos were thoroughly ground using SD-H₂O at a rate of 500mL per 150 g of embryos. The extract was then filtered under vacuum, and the filtrate was stored in dark-colored bottles in a 4 °C refrigerator. Another 72-h extraction was carried out using 70% methanol, and the leftover embryo residue was quantitatively transferred to alcohol. The alcohol was entirely evaporated from the filtrate by passing it through a rotary evaporator after filtering. MEE was produced by combining alcoholic extracts with water.

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 Se, AsA, and GA₃. 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 tomato treatments in this study (Table 3).

The extracts were stored in the refrigerator (-20°C) until needed, or they were used right away

2.5. Sampling

Nine randomly selected plants from each of the six treatments had their top third leaf collected when the plants were 60 days post-transplant. As soon as the samples were collected, they were taken to the lab to be analyzed for physio-biochemical indices and growth components. Fruit yield and quality components were assessed once the fruits of the plants had reached full maturity.

2.6. Estimation of photosynthesis-related parameters

Total leaf contents (mg g⁻¹ fresh tissue) of chlorophyll a and chlorophyll b, and carotenoids were estimated by applying procedures described by Wellburn, [35]. SPAD chlorophyll index was evaluated using the Minolta chlorophyll meter (Osaka, Japan), while photosynthesis efficiency was assessed as Fv/Fm [36]. The performance index (PI) of photosynthesis was computed referring to Clark et al. [37]. Photochemical activity was evaluated following Jagendorf, [38] and Avron,[39] methods. To assess water use efficiency (WUE), μM and CO₂ assimilated mM⁻¹ H₂O transpired. For this, five leaves (five replicates) were subjected to gas exchange indices evaluations following a 10-to 15-minute acclimatization period in the leaf-to-leaf chamber. The evaluations were recorded at 1200 μmol m⁻² s⁻¹ PPFD, 380 μmol mol⁻¹ CO₂, 26±1 °C block temperature, and 60±5.0% relative humidity (RH). Furthermore, the intercellular CO₂ concentration (C_i; μmol CO₂ M⁻¹ air) was assessed, and WUE was calculated as the net assimilation rate divided by the transpiration rate.

2.7. Estimation of leaf nutrient contents

After the samples were digested in a 1 HClO₄: 3 HNO₃ (v/v) mixture, P, N, and K contents (mg g⁻¹ dry weight (DW)) were determined according to Page et al. [40], Jackson, [41], and A.O.A.C. [42], respectively. In addition, the contents of Fe, Mn, and Zn (mg g⁻¹ DW) were also determined, using atomic absorption spectroscopy, and standard reference samples (NIST, USA) were utilized [43]. Se (mg g⁻¹ DW) was quantified by following the protocol of Olson et al.[44].

2.8. Evaluation of leaf integrity

Using the protocols of Osman and Rady, [45]; Rady, [46]; and Rady and Rehman, [47], the leaf relative water content (RWC; %), membrane stability (MSI; %), and electrolyte leakage (EL; %) were measured. The equations below were utilized:

$$RWC (\%) = \left[\frac{(\text{fresh mass} - \text{dry mass})}{(\text{turgid mass} - \text{dry mass})} \right] \times 100$$

$$MSI (\%) = \left[1 - \left(\frac{EC1}{EC2} \right) \right] \times 100$$

MSI estimation is based on measuring the electrical conductivity (EC) of the tissue solution when it is warm (40 °C) (EC1) and when it is boiling (100 °C) (EC2).

$$EL (\%) = \left[\frac{(EC2 - EC1)}{EC3} \right] \times 100$$

EL estimation is based on measuring the EC of the tissue solution when it is normal (EC1), warm (45–55 °C) (EC2), and boiling (100 °C) (EC3).

2.9. Estimation of growth and yield traits

Samples of fruit and plants (45 days old) were collected from each treatment for every experiment. Following transplantation, the number of leaves on each 45-day-old plant was counted, and the leaf area was measured using a LI-3000C leaf area meter (Portable, LICOR Inc., Lincoln, NE, USA). Next, the plants were separated into roots and shoots. They used an electric balance to record their fresh weight (FW), and a 30 cm graduated ruler to measure their lengths. The dry weight (DW) of the plants was measured after they were dried at $70 \pm 2^\circ\text{C}$ until constant weights were reached. Upon harvesting, the number of fruits plant⁻¹, the total weight of fruits plant⁻¹, and the average weight of fruits were assessed.

2.10. Estimation of tomato fruit quality

Tomato fruit quality was assessed using homogeneous fruits that were selected from the first harvest time. Fruit total soluble solids (TSS) was measured using a digital refractometer (PR-100, Atago Co. Ltd., Tokyo, Japan) and the results were expressed in °Brix according to A.O.A.C. [42]. Vitamin C content in fruits was determined using the method of Okamura [48], which modified by Law et al. [49]. Organic acids and firmness were measured as described by Shao et al. [50]. Fruit lycopene content was determined using the method outlined with Sharma and Le Marguer, [51]. Fruit β-carotene content (mg kg⁻¹ fresh weight (FW)) was evaluated by exploiting the standard method of analysis included in the A.O.A.C. [42]. Fruit content of Se was determined according to the procedures of Pequerul et al. [52] and A.O.A.C. [42].

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 [53]. 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 [54].

3. Results

According to Table 1, in an initial trial aimed at determining the optimal concentrations of MEEst (a natural extract derived from maize grain embryos enriched with GA₃, AsA, and Se), it was found that the best results for tomato growth exceeding 22.5% concentration were obtained with MEEst at concentrations of 7.5 and 15.0%. Furthermore, MEEst was higher than all concentrations at 7.5 and 15.0%; 7.5–30% of un-enriched 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 WL, whereas 40% SRWC did so. Thus, in the primary trial, 60% SRWC was applied as WL. Subsequently, a primary investigation was conducted 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%.

It was discovered that: 7.5 and 15.0% MEEst treatments significantly improved all tested growth parameters, yield, and fruit quality, as well as photosynthesis measurements and nutrient contents. These findings came from a thorough verification of the main trials' results, which are shown in (Fig. 1-6). Additionally, under F-IV and WL treatments, there was a discernible preference for the 15.0% MEEst treatment over the 7.5% MEEst treatment. Overall, all the results show that, when applied under WL as opposed to F-IV, the 15.0% MEEst treatment is more effective.

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 ≤ 0.05. FW; fresh weight, DW; dry weight.

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 ≤ 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

3.1. Response of photosynthetic parameters and water use efficiency (WUE) of tomato to SRWC and MEEst

Chlorophyll a, chlorophyll b, carotenoids contents, SPAD chlorophyll index values, Fv/Fm, PI, photochemical activity, and WUE were all significantly reduced by WL, as shown in (Fig. 1), by 66.4%, 55.9%, 49.6%, 21.7%, 21%, 19.6%, 26%, and 30%, respectively, in comparison to those obtained from corresponding control (F-IV). In comparison to the corresponding control (F-IV), the 15.0% MEEst increased the following in fully irrigated plants: chlorophyll a by 76%, chlorophyll b by 50%, total carotenoids by 87%, SPAD chlorophyll index by 19%, Fv/Fm by 12%, PI by 76%, photochemical activity by 24%, and WUE by 24%. With respect to the corresponding stressed control, 15.0% MEEst enhanced the levels of chlorophyll a, chlorophyll b, total carotenoids, SPAD chlorophyll index, Fv/Fm, PI, photochemical activity, and WUE in deficit-irrigated plants by 162%, 72%, 54%, 14%, 27%, 20%, 38%, and 54%, respectively.

3.2. Response of nutrient contents of tomato to SRWC and MEEst

Significant reductions in macro- and micronutrient contents were observed in WL. Compared to fully irrigated plants (F-IV), the reductions were 49% for N, 47% for P, 51% for K, 38% for Fe, 41% for Mn, 45% for Zn, and 30% for Se (Fig. 2). In comparison to the control (F-IV), 15.0% of MEEst significantly increased the contents of various nutrients under normal conditions. The increases were 24% for N, 34% for P, 29% for K, 24% for Fe, 29% for Mn, 35% for Zn, and 230% for Se. In comparison to the corresponding stressed control, the MEEst 15.0% treatment under WL showed higher nutrient contents by 97% for N, 94% for P, 107% for K, 65% for Fe, 66% for Mn, 87% for Zn, and 763% for Se. With the exception of Se, the maximum nutrient contents were attained under F-IV×15% MEEst treatment.

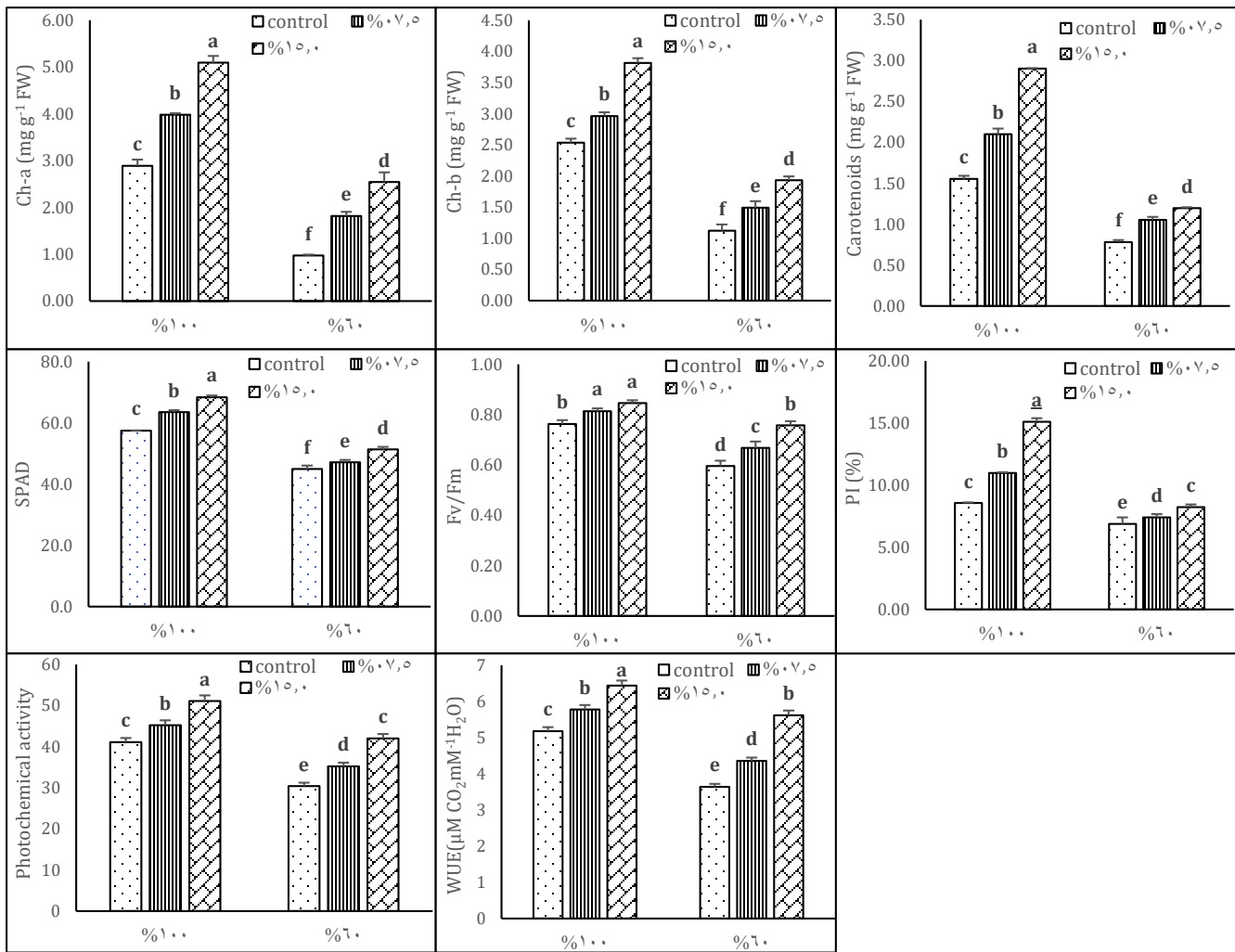


Fig. 1. Effect of exogenous application of maize grain embryos-derived natural extracts (MEE) enriched with some growth stimulators (MEEst) on photosynthetic parameters of tomato (*Solanum lycopersicum* L. cultivar 023) plants grown under two irrigation regimes (IR; 100 and 60% of soil water holding capacity). Values are means \pm 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 $P \leq 0.05$. SPAD value; Soil Plant Analysis Development; This numerical SPAD value specifies the relative content of chlorophyll within the leaf sample, Fv/Fm; photosystem II quantum efficiency, PI; performance index, and PhChem activity; Photochemical activity, WUE; water use efficiency.

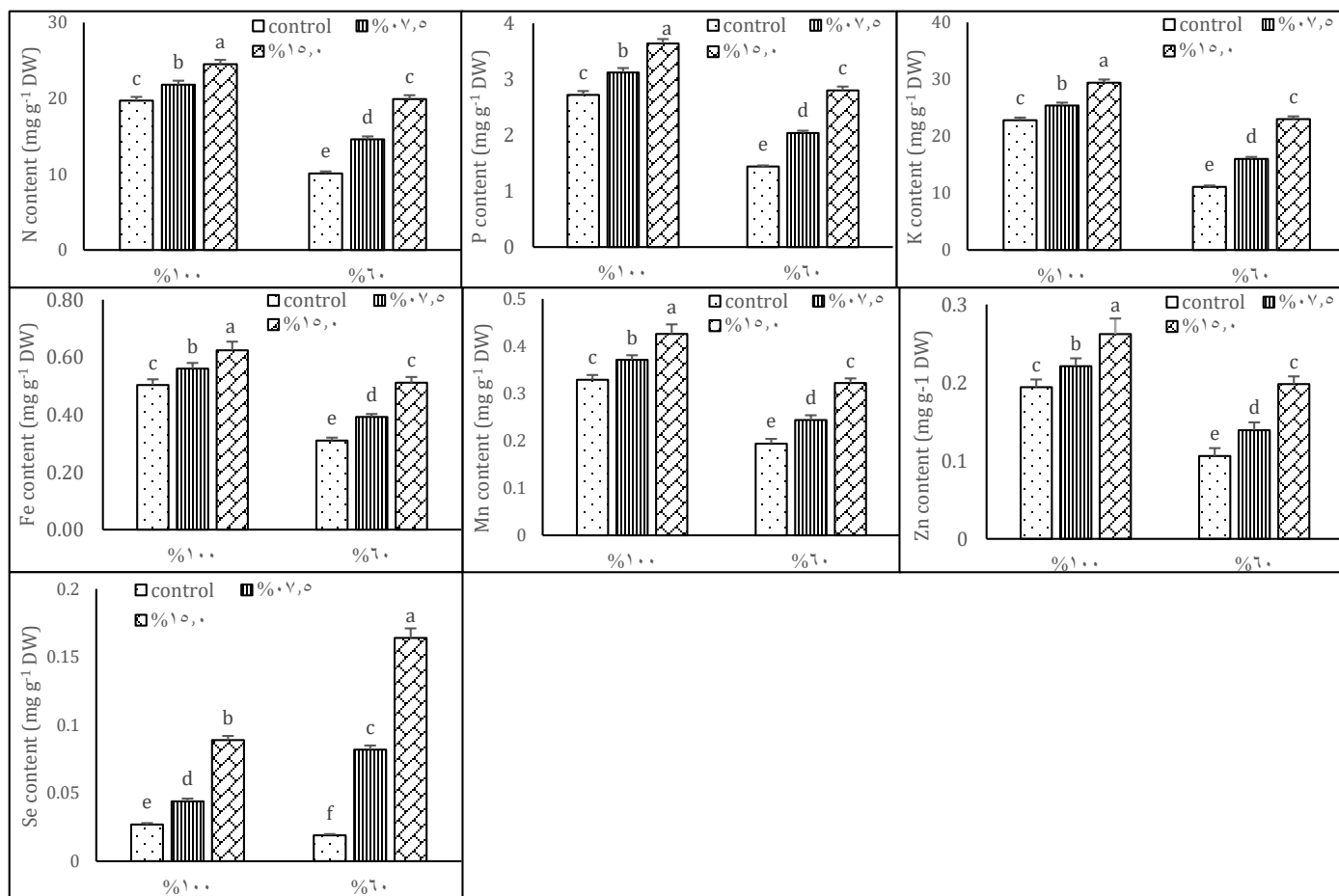


Fig. 2. Effect of exogenous application of maize grain embryos-derived natural extracts (MEE) enriched with some growth stimulators (MEEst) on nutrient contents of stomato (*Solanum lycopersicum* L. cultivar 023) plants grown under two irrigation regimes (IR; 100 and 60% of soil water holding capacity). 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 $P \leq 0.05$. N; Nitrogen, P; Phosphorus, K; Potassium, Fe; Iron, Mn; Manganese, Zn; Zinc, Se; selenium.

3.3. Response of leaf integrity of tomato to SRWC and MEEst

The measurements of tomato leaf integrity included EL, MSI, and leaf RWC (Fig. 3). When compared to irrigation with F-IV, the negative effects of WL-induced stress on *Solanum lycopersicum* L. plants were reported as increases in EL by 212%, decreases in RWC by 17%, and increases in MSI by 29%. In reference to the MEEst treatment, 15.0% MEEst reduced EL by 29% while increasing RWC and MSI by 21% and 7%, respectively, in comparison to untreated plants (control). The tomato plants supplemented with 15.0% MEEst showed a significant reduction in WL-induced damage to tissue stability, with improvements in RWC and MSI of 15% and 17%, respectively, and a 36% reduction in EL when measuring against stressed plants.

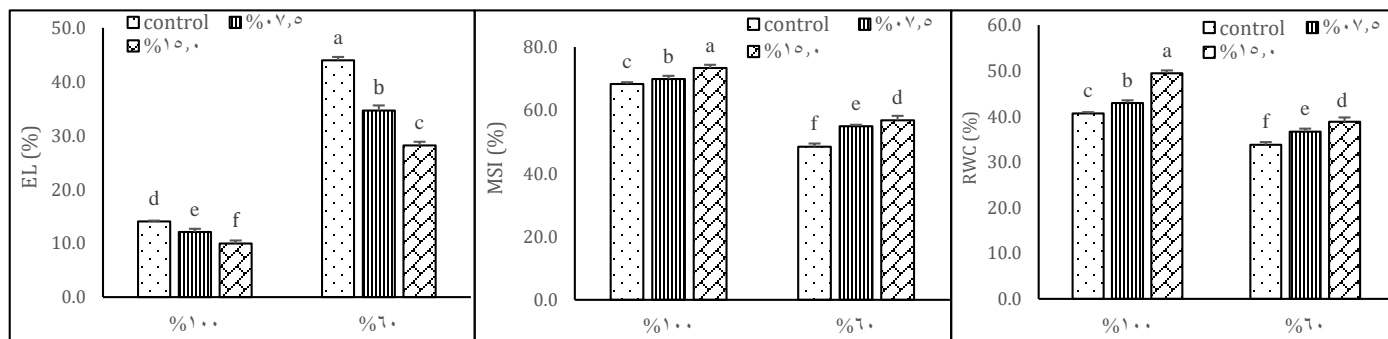


Fig. 3. Effect of exogenous application of maize grain embryos-derived natural extracts (MEE) enriched with some growth stimulators (MEEst) on leaf integrity in tomato (*Solanum lycopersicum* L. cultivar 023) plants grown under two irrigation regimes (IR; 100 and 60% of soil water holding capacity). 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 $P \leq 0.05$. EL; electrolyte leakage, MSI; membrane stability index, RWC; relative water content.

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

The findings depicted in (Fig. 4) demonstrate that the growth traits of *Solanum lycopersicum* L. plants (leaf area plant⁻¹, number of leaves plant⁻¹, shoot length plant⁻¹, root length plant⁻¹, shoot FW plant⁻¹, shoot DW plant⁻¹, root FW plant⁻¹, and root DW plant⁻¹) were significantly reduced by WL stress in comparison to the control 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 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.

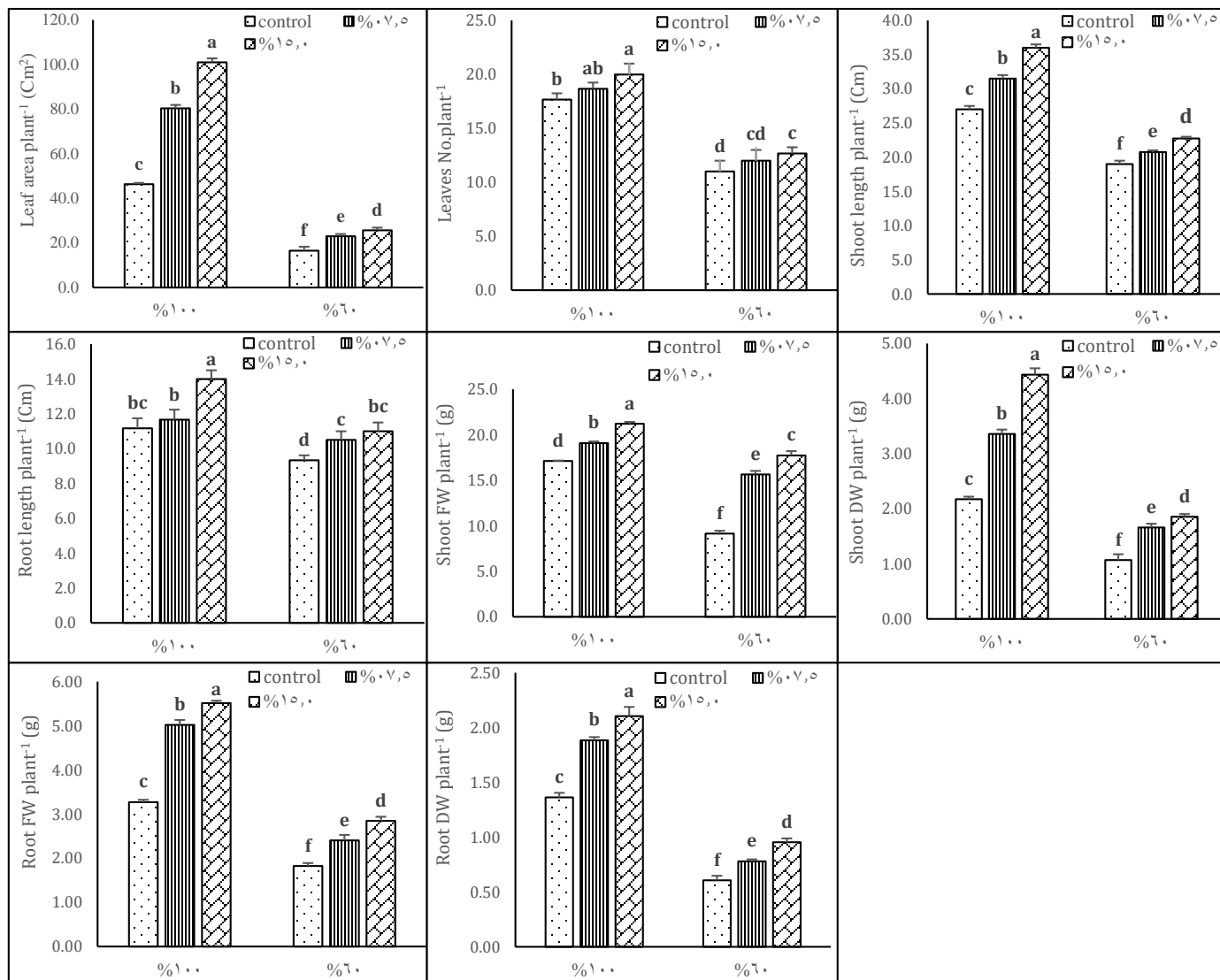


Fig. 4. Effect of exogenous application of maize grain embryos-derived natural extracts (MEE) enriched with some growth stimulators (MEEst) on growth traits of tomato (*Solanum lycopersicum* L. cultivar 023) plants grown under two irrigation regimes (IR; 100 and 60% of soil water holding capacity). Values are means \pm 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 $P \leq 0.05$.

3.5. Response of yield components of tomato to SRWC and MEEst

The yield components were affected by the effects of WL. As shown in (Fig. 5), comparing the irrigated plants with 60.0% evapotranspiration to the corresponding control (F-IV), the irrigation significantly reduced the yield parameters, such as fruits No. plant⁻¹, fruits total weight plant⁻¹, and a fruit average weight by 39%, 40%, and 49%, respectively. Nevertheless, when compared to the corresponding control, the treatment with 15.0% MEEst significantly increased the number of fruits per plant, fruits total weight plant⁻¹, and the average weight of fruits in the plants subjected to WL by 59%, 47%, and 76%, respectively, compensating for the yield reduction that had occurred. When 15.0% MEEst was applied to plants under normal circumstances, the plants' yield parameters-fruit number per plant, fruit total weight per plant, and fruit average weight-improved by 47%, 11%, and 72%, respectively, in comparison to the corresponding plants grown under full irrigation without the application of any MEEst (F-IV).

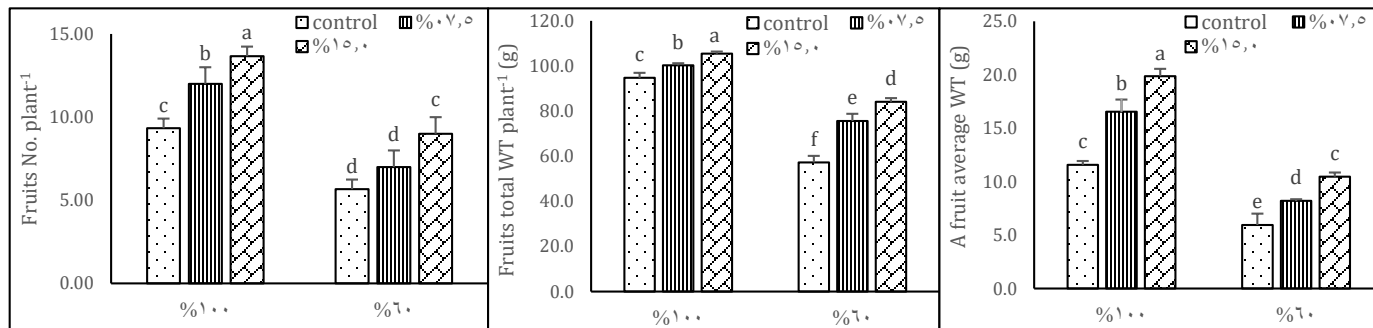


Fig. 5. Effect of exogenous application of maize grain embryos-derived natural extracts (MEE) enriched with some growth stimulators (MEEst) on yield of tomato (*Solanum lycopersicum* L. cultivar 023) plants grown under two irrigation regimes (IR; 100 and 60% of soil water holding capacity). Values are means \pm 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 $P \leq 0.05$.

3.6. Response of fruit quality traits of tomato to SRWC and MEEst

Fruit analyses revealed differences between the two irrigation regimes, as shown in (Fig. 6). In comparison to those obtained under F-IV, WL significantly decreased Se by 11% and noticeably increased (TSS, vitamin C, organic acids, lycopene, firmness, and β -carotene) by 21%, 13%, 29%, 21%, 13%, and 12%, respectively. In comparison to the corresponding control (F-IV), 15.0% MEEst demonstrated a significant increase in total soluble solids (TSS) of 11%, vitamin C of 11%, organic acids of 12%, lycopene of 2%, firmness of 12%, β -carotene of 11%, and Se of 26% under normal conditions. Under stress, 15.0% MEEst showed a significant increase over the corresponding stressed control in TSS by 13%, vitamin C by 16%, organic acids by 16%, lycopene by 14%, firmness by 15%, β -carotene by 11%, and Se by 23%.

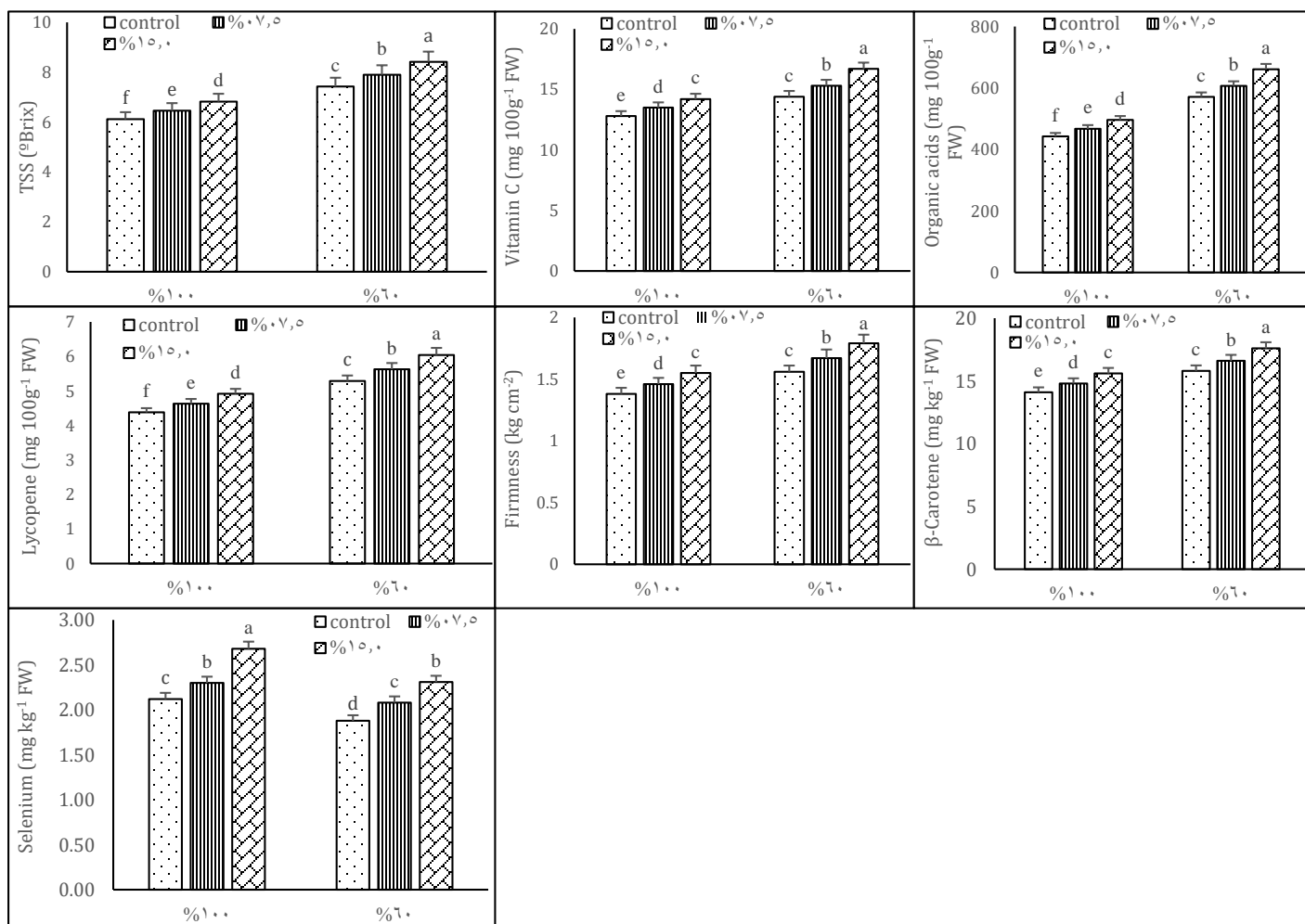


Fig. 6. Effect of exogenous application of maize grain embryos-derived natural extracts (MEE) enriched with some growth stimulators (MEEst) on fruit quality traits of tomato (*Solanum lycopersicum* L. cultivar 023) plants grown under two irrigation regimes (IR; 100 and 60% of soil water holding capacity). Values are

means \pm 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 $P \leq 0.05$.

4. Discussion

Previous research indicates that the natural extract (MEE) derived from maize grain embryos is very beneficial in mitigating the adverse effects of plant stresses, such as the stress brought on by low water availability (WL) [55, 33]. However, there is a small body of research that suggests using MEE enriched with biostimulators like polyamines (PAs) to treat tomato transplants to mitigate the harmful effects of WL [30]. Moreover, no studies on the application of MEE enriched with biostimulators like GA_3 , AsA, and Se (MEEst) to tomato transplants have been planned yet. In this study, treatment with 15.0% MEEst significantly outperformed 7.5% MEEst in terms of improvements in the growth, production, and fruit quality of tomato transplants grown under WL. Numerous studies of [12, 30, 56] have clarified significant alterations in the morpho-physio-biochemical and various components (e.g., enzymes, low molecular mass antioxidant compounds, and osmo-protectant compounds) of the antioxidant defence system of various plants grown under WL. In this report, positive changes were observed with up-regulatory assessment of photosynthesis and WUE (Fig. 1), nutritional status (Fig. 2), and leaf integrity (Fig. 3), resulting in satisfactory tomato growth and high-quality yield under WL (Fig. 4-6). This was attributed to the treatment of tomato plants with 15.0% MEEst.

In this study, tomato plant growth, yield, and fruit quality were severely harmed by WL stress, defined as a water deficit to 60% of SRWC. The growth, yield, and fruit quality of tomato plants were severely impacted (Fig. 4-6) by the WL treatment (60% SRWC), which also had a negative impact on photosynthetic pigments (chlorophylls and carotenoids), photosynthetic efficiency, nutritional balance, and leaf integrity (high ion leakage; EL, along with low MSI and RWC). As markers of oxidative stress, the excessive rise in superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) levels ensured these unfavorable results. Alharby et al. [30] reported that WL resulted in the closure of leaf stomata, a worsening of EL and MDA, and a significant reduction in PSII effectiveness (Fig. 1). Additionally, WL altered the typical tendency of components of the antioxidant defence system, and ultimately, the plant did not survive. On the other hand, this study's WL revealed that spraying tomato plants with MEEst had important advantages. 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.), PAs (i.e., spermine, spermidine, and putrescine), phytohormones (CKs, Z-CK, salicylic acid, etc.), and nutrients (N, P, K, etc.) can be used to secure the high benefits of MEEst. Together with the GA_3 , AsA, and Se enrichments that were secured for the MEEst tested in this study.

Plants damaged by stress responded better to the enriched MEEst than to the unenriched MEE (Table 3). Numerous studies of [57-61] have documented a positive correlation between enhanced photosynthetic efficiency, RWC, and WUE under WL and improved plant growth (biomass), yield, and quality. Our findings also investigated this beneficial relationship. Tomato transplants treated with MEEst under WL had strong growth, high production, and fruit quality comparable to those grown under non-stress conditions without MEEst treatment, thanks to the noticeable improvement in photosynthesis under WL (Fig. 1 and 4-6). This is because MEEst treatment (Table 3) allowed tomato transplants to grow strongly after WL and function normally (Fig. 4). The photosynthesis efficiency was restored under WL by MEEst treatment to restore damaged components (e.g., carotenoids, chlorophyll, Fv/Fm, PI, and photochemical activity, Fig. 1) and WUE.

Transplants enriched with MEEst, such as GA_3 , AsA, and Se (Table 3), accelerate metabolic processes and confer powerful growth to tolerate WL during spraying. According to the report's exploration, under WL MEEst, pigments related to photosynthesis and other indicators of photosynthesis efficiency can be preserved. This has a positive impact on plant WUE (Fig. 1) and has been shown to support reasonable growth and productivity, both quantitatively and qualitatively (Fig. 4-6).

In our study, WL stress reduced the amounts of carotenoids, chlorophyll a, and chlorophyll b in the leaves of tomato plants, as well as their photochemical activity, photosynthetic efficiency (Fv/Fm and PI), and SPAD chlorophyll index values (Fig. 1). Because of the plant's dependence on water and nutrients, leaf chlorophyll is one of the most significant physiological markers of stress [62, 63].

Furthermore, under WL stress, a strong correlation was observed between the tomato's SPAD chlorophyll index and total chlorophyll concentration [64]. Chlorophyll levels in WL-stressed plants may have decreased because of peroxidase enzymes and the synthesis of phenolic components [64], thylakoid membrane disarray, more chlorophyll degradation than synthesis via the formation of proteolytic enzymes like chlorophyllase, which is responsible for the degradation of chlorophyll, and damage to the photosynthetic apparatus [65]. Because of the observable sensitivity to stress and/or other damage caused by PSII complexes, Fv/Fm is frequently considered as an indicator of photo-inhibition. Fv/Fm gradually decreased under WL stress, indicating that WL was the cause of the PSII reaction center's closure. This limited electron transfer decreased the amount of light energy available for the PSII reaction center's actual photochemical reactions [66]. The application of MEEst to non-stressed plants reduced WL stress and improved photosynthetic machinery performance (Fig. 1). Because minerals are present in MEEst (Table 3) and may play a role in chlorophyll structure, there may be a decrease in the enzyme chlorophyllase's ability to degrade chlorophyll and an increase in chlorophyll biosynthesis. MEEst also increased the production of CKs, which encouraged the biosynthesis of chlorophyll.

Furthermore, MEEst has been shown to enhance the synthesis of carotenoids in well-watered and WL stressed plants. This protective effect on the photosynthesis system against ROS overproduction is achieved through upregulating the activities of pigment-synthesizing enzymes, which in turn reduces enzyme degradation [33]. Carotenoids are antioxidant compounds that shield the photosynthetic apparatus from photo-inhibitory damage by single oxygen and quench the excited triple state of chlorophyll. These compounds may be closely linked to the improved plant tolerance against WL [55].

An important strategy used by plants to withstand WL is the coordination of the relationship between carbon assimilation and water consumption, which controls changes in WUE by stressed plants [66]. In this study, WL stress caused a decrease in tomato leaf WUE, but MEEst treatment increased it in both stressed and unstressed plants (Fig. 1). The bioactive stimulants in MEEst that improved cell metabolism could be the cause of this.

When tomato plants are exposed to WL, a decrease in leaf RWC of their leaves indicates a decrease in relative water absorption or water maintenance. Furthermore, turgor pressure and plant size were lowered in plants under WL by lowering WUE and RWC. Consequently, it could be the cause of the decrease in tomato plant leaf area under WL stress (Fig. 4). The findings by Behboudi et al. [64] were comparable. In contrast to untreated stressed plants, MEEst-treated plants exposed to WL stress or well-watered were able to maintain their MSI and retain higher WUE and RWC (Fig. 1 and 3).

During development, it is critical that plants carry out their basic and routine cellular functions. Plants lacking nutrients due to the osmotic effects of water-logging stress and/or soil-logging stress cause disruptions in nutrient availability, uptake, translocation, and metabolism [67]. This results in a decrease in the amounts of macro- and micronutrients in tomato plants under water-logging stress (Fig. 2). Our findings indicate that applying MEEst considerably enhanced and restored the mineral nutrients in plants (Fig. 2). The fact that MEEst is rich in mineral nutrients and has enhanced absorption due to an increase in osmo-protectants may be the cause of this increase in the acquisition of minerals. Furthermore, the primary cause of this regulation in ionic uptake may be attributed to the MEEst-treated plant's increased cell MSI (Fig. 3), which in turn promotes ion uptake and transport selectivity.

Our findings from this investigation showed that, in contrast to plants that received an appropriate water supply, WL inhibited plant growth and total chlorophyll content. According to Ibrahim and Ibrahim, [68], WL has been shown to cause stomatal closure, which raises lipid peroxidation and releases an excessive amount of ROS [69], which resulted in the direct inhibition of chlorophyll degradation and photosynthesis [70]. Due to metabolic process disorders and an elevated rate of respiration brought on by the increased energy requirements, meristem and cell expansion activities were reduced, resulting in restricted tomato growth and yield components [71]. The internal antioxidant systems of tomato plants were strengthened by MEEst treatment in order to withstand these undesired results of WL stress and maintain plant life. These well-watered and stressed plants' improved growth and productivity are typically indicated by various cellular changes, such as improved photosynthetic pigment content (Fig. 1), increased water content and cell membrane stability (Fig. 3) and maintained ionic balance (Fig. 2). This may also be attributed to the abundance of phytohormones, auxins, CKs, including zeatins, gibberellins, and cytokinins in MEEst, particularly following its enrichment with (AsA, GA₃, and Se) (Table 3). Additionally, under various stresses, the MEEst bioactive components are crucial for promoting plant growth and development, including cellular enlargement and apical meristem division [32]. It is therefore important to note that MEEst is crucial for improving plant physiology during WL and causing a healthy metabolic state, which in turn promotes healthy plant growth and development (Fig. 4). Yildiztugay et al. [72] found a correlation between the water content and biomass of the plant. Our findings also showed this relationship (Fig. 1, 3 and 4-6).

When compared to well-watered plants, the fruit yield of tomato plants was lower in the WL plants. These findings may be explained by the fact that WL can limit photosynthesis by interfering with CO₂ assimilation in the Calvin cycle, stomatal conductance, and adenosine triphosphate (ATP) supply. It can also prevent ribulose 1,5-bisphosphate from regenerating [73]. Furthermore, the current investigation verified that WL impeded tomato plants' vegetative growth (Fig. 4) and adversely impacted the total chlorophyll (Fig. 1). Conversely, under WL conditions, there were notable increases in the concentrations of β -Carotene, firmness, organic acids, vitamin C, and TSS (Fig. 6). This could be because of the concentration effect, which is the reduction of water content in fruits that causes an increase in the concentration of the internal components. Furthermore, by influencing the soluble sugars in tomato fruits, the steady increase in (abscisic acid) ABA, which is thought to be a common indicator of WL stress in a number of plants, may improve tomato fruit quality [74]. Nevertheless, since ABA and carotenoids share the same biosynthetic pathway, rising lycopene under WL may also be connected to alterations in ABA [70]. Numerous studies have previously confirmed this improvement in tomato fruit quality under WL regarding TSS, vitamin C, organic acids, lycopene, firmness, and β -Carotene [75].

Because both well-watered and stressed plants in the current study had many catalysts from the MEEst treatment, applying MEEst to tomato plants increased plant growth and, ultimately, fruit yield and quality (Fig. 4-6). It is essential to plant growth and productivity because it increases photosynthetic efficiency, stomatal opening, osmoregulation, membrane stability, assimilating transport from source to sink, enzyme activity, and carbohydrate synthesis. Moreover, these positive results may be the result of improved nutrient absorption [55].

5. Conclusions

Because water limitation stress has negative effects on chlorophyll content, photosynthetic efficiency, water content, membrane lipid peroxidation, and electrolyte leakage because of increased oxidative stress, tomato plant growth and yield have severely decreased. One of the ways to mitigate these adverse effects is to use a plant extract as a transplant spraying strategy, such as biostimulants-rich MEE enriched with GA₃, AsA, and Se (MEEst). By strengthening the antioxidants, osmoregulation, and photosynthetic systems, the MEEst (15.0%) treatment increased tomato plant growth and yield. This resulted from decreased oxidative stress damage. The decrease in membrane lipid peroxidation and the electrolyte leakage under WL stress were indicative of all these favorable outcomes. According to this study, to develop WL-tolerant plants for efficient sustainable agriculture, plant extracts with signaling networks that interfere with numerous physiological and biochemical pathways, such as biostimulants-rich MEE enriched with GA₃, AsA, and Se, are predicted to exist.

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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