Saudi Journal of Biological Sciences 28 (2021) 6133–6144

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Using some growth stimuli, a comparative study of salt tolerance in two tomatoes cultivars and a related wild line with special reference to superoxide dismutases and related micronutrients

Hesham Faisal Alharby

Department of Biological Sciences, Faculty of Science, King Abdulaziz University, 21589 Jeddah, Saudi Arabia

ARTICLE INFO

Article history:
Received 21 May 2021
Revised 20 June 2021
Accepted 21 June 2021
Available online 25 June 2021

Keywords:
Antioxidative defense system
Growth promoters
Salinity
Solanum lycopersicum (L.)
Superoxide dismutases

ABSTRACT

Salinity is a major global problem that threatens the agricultural sector, especially in areas that suffer from a shortage of water. It motivates ionic toxicity, osmotic and oxidative stresses, which greatly inhibits plant performances and crop productivites. However, micronutrients (MNs) or plant extracts, like germinated maize grain extract (gMGE), have been reported to minimize the effects of salt stress on plant growth and returns. Therefore, this study aimed at evaluating the influences of MNs or gMGE applied as foliar sprays on growth, physio-biochemical indices, and antioxidative system components in three genotypes of tomato plants stressed by 9 dS m^{-1} NaCl. This salinity level markedly increased Na^+ content, lipid peroxidation, ion leakage, and markers related to oxidative stress (superoxide; $O_2^{\cdot-}$ and hydrogen peroxide; H_2O_2). Besides, marked increases in activities of enzymatic (especially different forms of superoxide dismutase; SODs) and non-enzymatic antioxidants and osmoprotectant compounds were also observed. In contrast, growth, photosynthetic capacity including hill reaction activity (HRA), K^+/Na^+ ratio, tissue cell integrity (e.g., cell water content and membrane stability), and K^+ and MNs contents decreased significantly under stress. However, compared to MNs, gMGE significantly improved the activities of the antioxidative system components (particularly SODs) and osmoprotectants, which were reflected in reduced Na^+ accumulation, lipid peroxidation, ion leakage, and oxidative stress. These results were coupled with remarkable elevations in photosynthetic capacity including HRA, K^+/Na^+ ratio, tissue cell integrity, K^+ content, and MNs contents, all of which were reflected in the enhancement of plant growth. Compared to local tomato cultivars (e.g., Castle Rock and C10), the wild line "0043-1" had better results. The interaction of three factors; salt stress, promoters, and tomato genotypes was significant. The wild tomato line "0043-1" as the best salt-tolerant is a good candidate for implication in breeding programs for tolerance to salinity to produce salt-tolerant cultivars for use to maximize tomato growth and productivity in saline environments.

1. Introduction

Among the vegetable crops, tomato (*Solanum lycopersicum* L.) is a master one that grows globally in both open field and controlled greenhouses. In most countries, the master healthy components for daily meals include tomato fruits, which are a rich source of many antioxidant compounds, including vitamins and minerals (Naika et al., 2005). However, tomato growth and yield have been reported to decrease considerably due to high salinity (Rady, 2011a, 2012; Zhang et al., 2016; Al-Daej, 2018; Singh et al., 2020). Although their response to salt stress is variable according to cultivar or line (Shannon et al. 1987), tomatoes are sensitive or moderately-tolerant to salt stress (Frary et al., 2010) through regulating ionic and water balance (Martinez-Rodriguez et al., 2008).

Salinity is a widespread abiotic stress and assassinates large areas of the agricultural land nowadays, especially in dry regions, including Saudi Arabia and Egypt. It is a major global problem that threatens the agricultural sector, as it inhibits plant growth and development, and deactivates plant physio-biochemistry through

E-mail address: aHeshamfasial@gmail.com
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.06.062
1319-562X/

osmotic stress and ionic cytotoxicity and thus impairs crop returns (Abd El-Mageed et al., 2018, 2020; Munns et al., 2006; Rady et al., 2019a). Worldwide, approximately 20% and 33% of non-irrigated and irrigated cultivated land, respectively, have been severely affected by increased salinity. By 2050, it is expected that arable land that will be affected by salt will reach more than 50% (Jamil et al., 2011; Shrivastava and Kumar, 2015). The harmful effects of saline stress have been proven in most crop plants, including tomatoes, where Na^+ and Cl^- ions have been notified to create hyperosmotic, hyperionic, and oxidative stress, affecting plant growth, physiological and biochemical indicators, and reproductive development, and plant death may be inevitable (Al-Daej, 2018; Yang and Guo, 2018; Rady et al., 2019b; Frukh et al., 2020). The production of excess reactive oxygen species (ROS; O_2^- and H_2O_2) and their outcomes such as excess lipid and protein oxidation have been used as biomarkers of oxidative stress in stress-suffered plants (Anjum et al., 2015; Rady et al., 2019c, 2020). To metabolize, combat, and control the accumulated ROS under stress, plants possess an efficient antioxidant defense system, which includes low molecular mass and enzymatic antioxidant compounds (e.g., proline, glutathione, ascorbate, superoxide dismutase, catalase, glutathione reductase, ascorbate peroxidase, etc.) (Frukh et al., 2020; Rady et al., 2019b, 2020). Besides, the elevated osmoregulation process through increased levels of glycine betaine, soluble sugars, ionic K^+, and proline gives plants additional mechanisms to withstand stress (Rady et al., 2019a, 2019b, 2019c). Although reports have reported regulation of cellular oxidative damage by components of the antioxidative system in stress-suffered plants (Anjum et al., 2015; Rady et al., 2019a; Frukh et al., 2020), the ability of the endogenous antioxidative system is not sufficient for plants to defend under acute conditions of environmental stressors. Thus, reports have recommended using exogenous helpful applications such as micronutrients (Abou El-Nour, 2002; El-Fouly et al., 2011; Noreen et al., 2018) and plant extracts, including the extract created from germinated maize grains (gMGE) to increase the plant's ability to withstand different stresses (Semida and Rady, 2014; Rehman et al., 2018; Alzahrani and Rady, 2019), including salt stress (Semida and Rady, 2014; Rady et al., 2019b).

Micronutrients (MNs) play key roles in plants for normal growth, health, and production sustainability under normal and abnormal environmental conditions. (El-Fouly et al., 2011; Noreen et al., 2018). Key MNs like Mn, Fe, Cu, and Zn are of equal importance in plant growth and development functions. They stimulate tolerance to environmental stressors throughout the life of the plant (Noreen et al., 2018). Additionally, they take part in enormous functions in plants, for example, they enter as functional components of many enzymes (Fe, Zn, and Cu) or activate many enzymatic reactions (Mn). They also play key roles in redox reactions of respiration (Fe) and photosynthesis (Fe and Mn), plant metabolism (Cu and Zn), and regulation of plant growth (Zn) (Kabata-Pendias and Pendias, 1999; Noreen et al., 2018).

Not so long ago, natural extracts as germinated maize grain-derived biostimulants (gMGE) have been applied to seeds or plants and reported to minimize the adverse stress effects on plant performances such as salinity (Semida and Rady, 2014; Fahad et al., 2014a; Fahad et al., 2014b ; Rady et al., 2019b), nutritional deficiency (Rehman et al., 2018), and the heavy metal cadmium (Alzahrani and Rady, 2019). The gMGE is rich in plant hormones (e.g., gibberellins, auxins, cytokinins, including zeatin-type cytokinin, etc.), osmoprotectant compounds (e.g., soluble sugars, free proline, glycine betaine, etc.), antioxidants (e.g., free proline, glutathione, ascorbate, etc.), and essential micronutrients (e.g., Fe, Mn, Zn, Cu, etc.). All gMGE-containing biostimulants are able to modify plant morphology, biochemistry, physiology, and antioxidant defense system to stimulate tolerance in plants to stress conditions, including salinity. Based on this information, little work has been implemented applying the effective gMGE-containing biostimulants as an innovative strategy to plants to withstand stress, including salinity (Semida and Rady, 2014; Rady et al., 2019b).

Therefore, plants from three genotypes of tomato (e.g., Castle Rock and C10 as local tomato cultivars *Solanum lycopersicum* L., along with line 0043–1 as an accession of the wild species *Solanum peruvianum* L.) were foliar-sprayed with gMGE (as a source of organic biostimulants) in comparison to foliar spray with a mixture of MNs (Fe, Mn, Zn, and Cu as a source of chemical growth stimulants). Compared to gMGE, MNs were utilized in this investigation due to their importance as key components of different superoxide dismutase forms and are important components in gMGE (Table 1). Possible positive modifications of biochemical and physiological systems of tomatoes were evaluated under the effects of foliar applications with MNs or gMGE to salt-stressed plants to identify the relationship between alterations in antioxidative defense system components and the range of plant tolerance, with respect to plant growth improvements.

2. Materials and methods

2.1. Preparation plant material for study

Seeds of Castle Rock and C10 (two local tomato cultivars *Solanum lycopersicum* L.) and of line 0043–1 (an accession of the wild species *Solanum peruvianum* L.) were used for the current study. Salt responses of these three genotypes were evaluated to specify their salinity threshold and selecting the appropriate salt concentration for use in this study. The salinity concentration of 9 dS m^{-1} was specified to be used in this study because the following concentration (12 dS m^{-1}) was lethal for plants of Castle Rock genotype (Table 2). Seeds of the two local tomato cultivars were supplied from the Center of the Agricultural Research, Egypt and seeds of the wild species were obtained from the Tomato NBRP (National Bioresource Project), Japan. The seed surface was sterilized by rinsing in 2% solution of NaClO for 10 min, well washed with distilled water, and air-dried for two hours.

Table 1
Germinated maize grain extract (gMGE) content of osmoprotectants, antioxidants, phytohormones, and micronutrients (on a dry or fresh weight basis).

Component	Unit	Value	Reference
***Osmoprotectants and antioxidants*:**			
Soluble sugars	mg g^{-1} DW	186.5 ± 8.3	Irigoyen et al. (1992)
Proline		43.4 ± 0.7	Bates et al. (1973)
Glycinebetaine		8.64 ± 0.15	Grieve and Grattan (1983)
Ascorbate	μmol g^{-1} DW	5.26 ± 0.11	Huang et al. (2005)
Glutathione		2.05 ± 0.05	Paradiso et al. (2008)
DPPH radical-scavenging activity	%	84.6 ± 1.4	Lee et al. (2003)
***Phytohormones*:**			
Auxins	μg g^{-1} FW	2.13 ± 0.04	Lavrich and Hays (2007)
Gibberellins (GAs)		2.24 ± 0.05	
Cytokinins (CKs)		2.56 ± 0.05	
Zeatin-type-CK		1.21 ± 0.02	
***Micronutrients*:**			
Fe	mg g^{-1} DW	16.42 ± 0.51	Chapman and Pratt (1961)
Mn		8.10 ± 0.28	
Zn		6.14 ± 0.19	
Cu		4.08 ± 0.15	

Table 2
Salt tolerance evaluation in two tomato cultivars (CR and C10) and a wild tomato species (line 0043–1) using NaCl salt.

Cultivars or species	NaCl salt treatments				
	Dw	3 dS m^{-1}	6 dS m^{-1}	9 dS m^{-1}	12 dS m^{-1}
CR	4	4	2	1	0
C10	4	4	4	3	2
Line 0043–1	4	4	4	4	4

0 means seedlings dead, **1** means weak growth, **2** means moderate growth, **3** means good growth, and **4** means very good growth.
CR means Castle Rock, and **Dw** means distilled water.

2.2. Tomato transplant husbandry

Using a net greenhouse at King Abdulaziz University, the seeds of the three genotypes were sown in flats of 209-cell Styrofoam [25 cm^3 (2.6 cm × 2.6 cm × 7.0 cm) for inverted pyramidal cell) at a rate of one healthy sterilized seed per pyramidal cell, which was filled with the medium suggested by Rady and Rehman (2016). The medium consisted of crushed corn grains, vermiculite, and peat moss at a rate of 0.5, 1.0, and 1.5, respectively and enriched with humic acid at a rate of 250 mg L^{-1}. Sowing began on August 2019 for a period of five weeks to obtain transplants. During this period, the mean temperatures and relative humidity were 32/27 ± 3/2 °C (day/night, respectively) and 61–65%, respectively with approximately 12.5 h of solar radiation as a natural day-length. The Styrofoam flats were arranged on rails and rotated daily to avoid any positional bias. Using overhead irrigation system, the transplants were irrigated daily with water and a nutritive solution alternating. The nutritive solution was consisted of Mo, B, P, Mn, Cu, Zn, N, K, and Fe, at a concentration of 0.35, 0.88, 0.88, 0.88, 0.88, 0.88, 1.75, 1.75, and 1.75 mg L^{-1}, respectively (Rady and Rehman, 2016). After five growing weeks, the healthiest and standardized transplants were collected from the flats of each genotype for further use in the current study.

2.3. Growing conditions and experimental setup

A pot experiment, repeated three times, was conducted from September 10 to December 20 using a greenhouse. Using the selected transplants of the three genotypes, transplanting was conducted into black colored plastic pots (42 cm deep and 40 cm inner diameter). The weight of 18 kg of clean, ion-free sand was filled into each pot, which was transplanted with a one tomato transplant. All transplants were kept normally under no stress for two weeks until the root system was repaired and well-fixed in the medium. Then, to apply 18 treatments for another 7 weeks, the transplants were allocated to 3 replications per treatment and 6 pots were specified per each replication. The 18 treatments were represented by three factors. Salt stress (9 dS m^{-1} using NaCl salt) and no stress were the first factor, three tomato genotypes; castle Rock, C10, and line 0043–1 were the second factor, while the third factor represented the foliar-sprayed promoters; micronutrients (MNs) and the extract obtained from germinated grains of maize crop (gMGE; municipal genotype, Egypt).

All pots were category (e.g., genotype)-organized in the open greenhouse and the transplants/plants were preserved in natural climatic conditions [e.g., the mean temperatures and relative humidity were 32 ± 3 °C/27 ± 3 °C for day/night (approximately 12 h for each) and 62–66%, respectively, and the availability of sunlight inside the greenhouse was kept homogeneous, with an average radiation of 12 h]. Salt stress treatment was applied by adding NaCl salt to the nutritious solution (Hoagland and Arnon, 1950) until reaching the concentration of 9 dS m^{-1}. This salty nutritious solution was applied day after day, and to maintain this level of salt stress, the EC was monitored continuously throughout the trial period. A nutritious solution free of NaCl salt was used to irrigate stress-free plants day after day. The Hoagland's nutritious solution (pH 5.9), containing 1250 μM $Ca(NO_3)_2 \times 4\ H_2O$, 250 μM KH_2PO_4, 1250 μM KNO_3, 500 μM $MgSO_4 \times 7\ H_2O$, 2.4 μM $MnCl_2 \times 4\ H_2O$, 11.6 μM H_3BO_3, 0.08 μM $CuSO_4 \times 5\ H_2O$, 0.24 μM $ZnSO_4 \times 7\ H_2O$, 0.13 μM $Na_2MoO_4 \times 2\ H_2O$, and 22.5 μM Fe^{3+}-$EDTA^+$ was used. Using diluted H_2SO_4, soil pH was modified to be 6.0–6.2.

Foliar sprays were applied with MNs (consisting of Fe, Mn, Zn, and Cu at concentrations of 1.6, 0.8, 0.6, and 0.4 g L^{-1}, respectively) and gMGE (10 mL of stock solution L^{-1}) three times for stressful and non-stressful genotypes; at 2, 4, and 6 weeks after transplanting. Using a hand atomizer, MNs, gMGE and distilled water (for control plants) were foliar sprayed to run off (1 l of spray solution per 5 plants), and an appropriate surfactant (e.g., few drops of Tween-20) was applied for the spraying solutions. Since they generated the best responses, the MNs and gMGE concentrations, which applied three times, were chosen according to our initial study (data not shown). The pots were regulated in a Split-Split plot design. The systematic errors that may result from climatic fluctuations in the local environment were averted through rotating the pots day after day before watering throughout the experimental period.

2.4. Preparation of micronutrient solution (MNs) and germinated maize grain extract (gMGE)

$EDTA^+$-Mn, $EDTA^+$-Fe, $EDTA^+$-Cu, and $EDTA^+$-Zn were dissolved in concentrations of 1.6, 0.8, 0.6, and 0.4 g L^{-1} of distilled water, respectively. This MNs solution was prepared at the same time as spray applications were implemented.

As detailed in Rehman et al. (2018) and Alzahrani and Rady (2019) procedures, gMGE was prepared including a minor modification. Using *Zea mays* grains (municipal genotype, Egypt), the extraction was implemented using germinated embryos, which were separated from the grains after germination with wet cotton and clean clothes. The germinated embryos were ground using distilled water. Under vacuum, the aqueous solution was filtered and the filtrate was kept in a black bottle in a refrigerator (at 4 °C). Another extraction was performed using the residues and ethyl alcohol (95%) with an electric shaker for 24 h, and the alcoholic filtrate was obtained through filtration under vacuum. Using rotary evaporator, the alcoholic filtrate was evaporated to entirely remove alcohol. The mixture of aqueous + alcoholic extracts was concentrated to obtain the stock solution of gMGE (a total of 240 mL was extracted from 6 kg of maize grains). The gMGE stock solution was kept at - 20 °C or used immediately.

The gMGE was evaluated for its main components and the estimated results are shown in Table 1.

2.5. Sampling date and sample preparation for different determinations

Nine weeks after transplanting, tomato plants of the three genotypes were harvested from different treatments. Data are dis-

played as an average of three independent experiments. After cleaning plant shoots using water-filled bucket to remove any adhering dusts, morphological, physiological, and biochemical attributes, as well as antioxidant enzyme activities, including different forms of superoxide dismutase were assessed in five replicates using the topmost fully enlarged young leaves.

2.6. Assessment of growth traits

Five plants were randomly chosen and extracted from sand by using a bucket filled with water. After separating plants, roots and shoots were subjected for taking fresh weights (FW). Dry weights (DW) were assessed after drying at 70 °C. The DW was taken after two or three constant weights.

2.7. Assessment of leaf pigments, chlorophyll fluorescence, and parameters of gas exchange

Fresh tissue of a fully-extended upper leaf devoid of the midribs was extracted using 80% (v/v) acetone with clean mortar and pestle to determine chlorophylls and carotenoids (Arnon, 1949). Optical densities of supernatants were monitored using spectrophotometer apparatus at wavelengths of 663, 645, and 480 nm. Using the fully enlarged upper leaves, the fluorimeter apparatus (PAM-chlorophyll, Germany) was utilized to evaluate chlorophyll fluorescence components (Li et al., 2007). Using the same leafy material, the infrared gas analyzer apparatus (LCA-4 model, England) was utilized to evaluate each of net photosynthesis rate; *Pn*, CO_2 assimilation rate; *A*, conductance of leafy stomata; *gs*, and transpiration rate; *E*. The activity of the hill reaction (Giebel, 2006) was evaluated and the activity rate was expressed in mM 2, 6-dichlorophenolindophenol (DCPIP) reduced h^{-1} mg^{-1} chlorophyll.

2.8. Assessment of relative water content (RWC) and osmoprotectants contents

Using a fully-extended upper leaf devoid of the midribs, a constant number of 2 cm-diameter discs was specified to determine the relative water content (Osman and Rady, 2014). Immediately, the discs were weighed to record the fresh mass. Then, in the dark, the discs were watery-saturated for 24 h. After gentle removing the adhering water, the turgid mass was recorded. Then, the dry mass of the discs was taken after drying (at 70 °C for 48 h). The RWC percentage was calculated using the following equation:

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

Leaf proline content (μg g^{-1} FW) was determined, after extraction using toluene, taking the absorbance readings at 520 nm (Bates et al. 1973). The Grieve and Grattan (1983) method was applied to assess the content of glycine betaine (μg g^{-1} FW) by colorimetrically monitoring the periodide crystals (formed by the reaction of cold $KI^{-}I_2$ as a reagent with the reaction mixture) at 365 nm. The method of Irigoyen et al. (1992) was utilized for extracting (with C_2H_5OH, 96%) and determining sugar content (mg g^{-1} FW) by taking the absorbance readings at 625 nm after cooling the mixture reaction obtained from mixing the anthrone reagent with the ethanolic leafy extract.

2.9. Assessment of markers of oxidative stress (superoxide and hydrogen peroxide), peroxidation of lipids, ionic leakage (EL), and membrane stability index (MSI)

The methods described in Velikova et al. (2000), Kubis (2008), Madhava Rao and Sresty (2000), Dionisio-Sese and Tobita (1998), and Rady (2011b) were utilized to determine each of hydrogen peroxide; H_2O_2, superoxide; $O_2^{\cdot-}$, lipid peroxidation (evaluated as malondialdehyde; MDA) contents, ionic leakage (EL), and membrane stability index (MSI), respectively using a fully-extended upper leaf devoid of the midribs.

Using a fully-extended upper leaf devoid of the midribs, a constant number of 20 discs was specified to assess the total inorganic ions escaping from the leaves. The EC1 (electrical conductivity) was recorded in the solution of the discs before heating. The EC2 was recorded after heating on 45 °C – 55 °C for ½ h. Then, the EC3 was recorded after boiling for 10 min. The EL percentage was obtained using the following equation:

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

Using a fully-extended upper leaf devoid of the midribs, a constant weight of 0.2 g pieces to determine the MSI. The EC1 was recorded after heating the solution of 0.2 g sample on 40 °C for ½ h. The EC2 was recorded after boiling the solution of another 0.2 g sample for 10 min. The MSI percentage was obtained using the following equation:

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

2.10. Assaying of ascorbate (AsA) and glutathione (GSH) levels and enzymatic activity

Using a fully-extended upper leaf devoid of the midribs, a prepared homogenate was subjected to a centrifugation (4,000 × g, 20 min) to produce a supernatant that was utilized to assass the AsA content (Huang et al., 2005). The same leafy material was applied to determine the GSH content (Paradiso et al., 2008).

Using a fully-extended upper leaf devoid of the midribs (0.5 g), enzymatic extract was prepared and used as the supernatant obtained from the centrifugation (12,000 × *g*, 4 °C, 0.25 h) of the leafy homogenate to assay enzyme activities (Unit mg^{-1} protein). The Aebi (1984), Nakano and Asada (1981), and Foster and Hess (1980) methods were practiced to assay catalase, ascorbate peroxidase, and glutathione reductase activities, respectively.

2.11. Assessment of ion contents and superoxide dismutases assays

To analyze micronutrient (Fe, Mn, Zn, and Cu) contents, fully-extended upper leaf samples were dried at 70 °C until constant weights were reached. After grinding, a mixture of perchloric and nitric acids (at 1: 3, v/v, respectively) was applied to digest the dried samples. Micronutrient contents were measured using atomic absorption spectroscopy apparatus (Johnson and Ulrich, 1959). The same digested solution was applied to determine the contents of K^+ and Na^+ using atomic absorption spectrophotometry (Emilio et al., 1998).

A frozen sample (500 mg) was homogenized in a mortar and pestle fixed on ice. The solution of homogenization was 10 mL HEPES buffer (50 mM) and 0.l mM Na_2EDTA (pH 7.6). Centrifugation (15,000 × *g* for 15 min at 4 °C) of homogenates was practiced to obtain an extract that applied to assay protein and superoxide dismutase (SOD). Overnight, the extract was dialyzed against dilute homogenizing solution to separate the the low-molecular-mass substances that interfere in SOD assay. The protein–dye binding method of Bradford (1976) was used to measure soluble protein concentration.

The procedure of Yu and Rengel (1999) was practiced to assay the activity of SOD (EC 1.15.1.1). This procedure is based on observation of photochemical reduction inhibition of NBT (nitro blue

tetrazolium). To assay total SOD, 5 mL reaction mixture [50 mM HEPES (pH 7.6) + 0.l mM EDTA + 50 mM Na_2CO_3 (pH 10.4) + 13 mM methionine + 0.025% (w/v) Triton X-l00 + 75 μM NBT + 2 μM riboflavin + 0.2 mL enzyme extract] was prepared. Using an intensity of light (350 μM m^{-2} s^{-1}), the mixture was illuminated for 15 min. One unit of activity of SOD was specified as the enzyme quantity causing a 50% inhibition of NBT reduction as observed spectrophotometrically (560 nm). Based on the procedures of Giannopolitis and Ries (1977) and Yu and Rengel (1999), 3 mM KCN or 5 mM H_2O_2 was applied in the reaction mixture to assay the activity of different SOD forms. KCN has no inhibitory effects on Mn-SOD and Fe-SOD, while it has an inhibitory effect on Cu/Zn-SOD. On the other hand, H_2O_2 has inhibitory effects on Cu/Zn-SOD and Fe-SOD without affecting Mn-SOD. Additionally, peroxidases could be interfered with SOD assay in the existence of external H_2O_2 (Yu et al., 1998). Thus, before adding H_2O_2, KCN was added at a final concentration of 3 mM to inhibit peroxidases (Chen and Asada, 1989). The activity of Mn-SOD was assayed in the existence of 3 mM KCN and 5 mM H_2O_2. The activity of Fe-SOD was secured by subtracting the activity of Mn-SOD from the activity yielded in the existence of 3 mM KCN, and the activity of Cu/Zn-SOD was computed by obtaining the difference between total SOD activity and the activities of both Mn-SOD and Fe-SOD. To correct for background absorbance, identical not illuminated reaction mixtures were used (Yu and Rengel, 1999).

2.12. Experimental layout and data analyses

The experiments were regulated as a split-split in a completely randomized design with two salinity levels (no stress and salt stress using NaCl up to 9 dS m^{-1}), three tomato genotypes, and three foliar applications (distilled water, micronutrients, and maize grains extract) in 3 replications each with 6 pots. Data are displayed as mean values ± SE. The data were statistically analyzed using Statistica (version 9, Tulsa, OK, USA). Comparing the data was applied using two-way ANOVA, followed by Tukey's Multiple Comparison Test.

3. Results

3.1. Effects on indices of growth and photosynthetic efficiency

For salt stress, saline watering of tomato plants significantly decreased fresh and dry weights (FW and DW) of plant shoots and roots chlorophylls content, carotenoids content, PSII efficiency (Fv/Fm) and its quantum yield (FPSII), photochemical quenching (qP), and hill reaction activity by 31.3, 29.4, 32.9, 30.4, 31.2, 14.7, 4.9, 12.0, 13.3, and 27.3%, respectively, while significantly increased non-photochemical quenching (NPQ) by 24.4% compared to non-salty irrigation (Tables 3 and 4). For tomato genotypes, C10 and 0043–1 genotypes had significant increases in shoot and root FW and DW, chlorophylls content, carotenoids content, Fv/Fm, FPSII, qP, and hill reaction activity, while had significant reduction in NPQ compared to Castle Rock (CR) genotype, with wild genotype (0043–1) outperforming other local genotypes (CR and C10) for all of the growth traits mentioned above, except for shoot FW. The 0043–1 genotype significantly outperformed the CR genotype by 27.0, 48.3, 39.6, 50.0, 7.9, 21.9, 18.2, and 85.7% for shoot DW, root DW, chlorophylls content, carotenoids content, Fv/Fm, FPSII, qP, and hill reaction activity, respectively. Regarding the promoters' application, foliar application with micronutrients (MNs) and germinated maize grain extract (gMGE) resulted in significant increases in all growth indices comparing with the control (distilled water), with gMGE outperforming MNs for all the above mentioned growth traits. Foliar application with gMGE increased shoot FW by 19.5%, root FW by 20.0%, shoot DW by 27.7%, root DW by 26.5%, chlorophylls content by 30.1%, carotenoids content by 13.3%, FPSII by 8.8%, qP by 6.1%, and hill reaction activity by 37.5%, while decreased NPQ by 13.0% comparing with the control. For the interaction of the three factors; salt stress, tomato genotypes, and promoters' application, significant differences (except for Fv/Fm) were observed among the combined treatments for all of the above-mentioned indices of growth, leaf photosynthetic pigments, and chlorophyll fluorescence (Tables 3 and 4).

3.2. Effects on indices of gas exchange, and contents of leaf relative water and osmoprotectant compounds

Regarding salt stress, saline irrigation of tomato plants significantly reduced gas exchange traits [e.g., rate of net photosynthesis (*Pn*), transpiration rate (*E*) and CO_2 assimilation (*A*), and stomatal conductance (*gs*)] and relative water content (RWC) by 29.6, 30.0, 35.7, 34.7, and 24.6%, respectively, while significantly increased the contents of osmoprotectants like proline, soluble sugars, and glycine betaine (GB) by 39.4, 30.1, and 13.8%, respectively compared to non-salty irrigation (Tables 5 and 6). Concerning tomato genotypes, 0043–1 genotype had significant increases in all parameters of gas exchange and the contents of relative water and osmoprotectant compounds compared to C10 genotype, which in turn displayed significant increases in all of these parameters compared to CR genotype. The wild genotype (0043–1) had increases in *Pn*, *A*, *gs*, *E*, RWC, proline content, soluble sugars content, and GB content by 42.0, 54.5, 62.4, 69.6, 17.8, 25.7, 32.6, and 20.6%, respectively compared to CR genotype. Regarding the promoters' application,

Table 3
Impact of foliar application with micronutrients (MNs) or germinated maize grains extract (gMGE) on growth and biomass yield of a wild tomato species (line 0043–1) and two tomato cultivars [Castle Rock (CR) and C10] that differed in their tolerance to salinity and growing under adverse conditions of 9 dS m^{-1} NaCl.

Source of variation	Fresh mass of shoot (g)	Fresh mass of root (g)	Dry mass of shoot (g)	Dry mass of root (g)
Salinity (S)	*	*	*	*
- NaCl	39.3^{a} ± 3.8	18.0^{a} ± 1.6	4.17^{a} ± 0.36	2.14^{a} ± 0.19
+ NaCl (**9 dS m^{-1}**)	27.0^{b} ± 2.6	12.7^{b} ± 1.1	2.80^{b} ± 0.30	1.49^{b} ± 0.12
Cultivars/species (CS)	*	*	*	*
CR	28.4^{c} ± 2.7	12.3^{c} ± 1.1	3.00^{b} ± 0.27	1.45^{c} ± 0.12
C10	37.0^{a} ± 3.5	16.1^{b} ± 1.5	3.65^{a} ± 0.30	1.84^{b} ± 0.15
0043–1	33.9^{b} ± 3.3	17.6^{a} ± 1.5	3.81^{a} ± 0.32	2.15^{a} ± 0.20
Promoters (Pr)	*	*	*	*
Control (**without**)	30.2^{c} ± 2.9	14.0^{c} ± 1.2	3.07^{c} ± 0.30	1.62^{c} ± 0.12
MNs	33.1^{b} ± 3.2	15.2^{b} ± 1.2	3.48^{b} ± 0.32	1.77^{b} ± 0.16
gMGE	36.1^{a} ± 3.5	16.8^{a} ± 1.6	3.92^{a} ± 0.33	2.05^{a} ± 0.19
S × CS × Pr	*	*	*	*

* indicates differences at $P \leq 0.05$ probability level. In each column, means with different letters are considered significantly different.

Table 4
Impact of foliar application with micronutrients (MNs) or germinated maize grains extract (gMGE) on leaf pigments and fluorescence of chlorophyll of a wild tomato species (line 0043–1) and two tomato cultivars [Castle Rock (CR) and C10] that differed in their tolerance to salinity and growing under adverse conditions of 9 dS m^{-1} NaCl.

Source of variation	Chlorophylls (mg^{-1} FW)	Carotenoids (mg^{-1} FW)	Fv/Fm	FPSII	qP	NPQ	Hill reaction activity (mM DCPIP reduced h^{-1} mg^{-1} chlorophyll)
Salinity (S)	*	*	*	*	*	*	*
- NaCl	1.54^{a} ± 0.04	0.34^{a} ± 0.02	0.81^{a} ± 0.03	0.75^{a} ± 0.03	0.90^{a} ± 0.03	0.45^{b} ± 0.01	0.11^{a} ± 0.00
+ NaCl (**9 dS m^{-1}**)	1.06^{b} ± 0.02	0.29^{b} ± 0.01	0.77^{b} ± 0.02	0.66^{b} ± 0.02	0.78^{b} ± 0.03	0.56^{a} ± 0.01	0.08^{b} ± 0.00
Cultivars/species (CS)	*	*	*	*	*	*	*
CR	1.06^{c} ± 0.02	0.26^{c} ± 0.01	0.76^{b} ± 0.02	0.64^{c} ± 0.02	0.77^{c} ± 0.03	0.57^{a} ± 0.01	0.07^{c} ± 0.00
C10	1.35^{b} ± 0.04	0.31^{b} ± 0.02	0.79^{ab} ± 0.03	0.70^{b} ± 0.02	0.84^{b} ± 0.03	0.51^{b} ± 0.01	0.09^{b} ± 0.00
0043–1	1.48^{a} ± 0.04	0.39^{a} ± 0.02	0.82^{a} ± 0.03	0.78^{a} ± 0.02	0.91^{a} ± 0.03	0.44^{c} ± 0.01	0.13^{a} ± 0.01
Promoters (Pr)	*	*	ns	*	*	*	*
Control (**without**)	1.13^{c} ± 0.03	0.30^{b} ± 0.01	0.78^{a} ± 0.02	0.68^{b} ± 0.02	0.82^{b} ± 0.02	0.54^{a} ± 0.01	0.08^{c} ± 0.00
MNs	1.30^{b} ± 0.04	0.31^{b} ± 0.02	0.79^{a} ± 0.02	0.70^{b} ± 0.02	0.84^{ab} ± 0.03	0.51^{b} ± 0.01	0.09^{b} ± 0.00
gMGE	1.47^{a} ± 0.03	0.34^{a} ± 0.02	0.80^{a} ± 0.03	0.74^{a} ± 0.02	0.87^{a} ± 0.03	0.47^{c} ± 0.01	0.11^{a} ± 0.01
S × CS × Pr	*	*	ns	*	*	*	*

* indicates differences at $P \leq 0.05$ probability level and "ns" means not significant difference. In each column, means with different letters are considered significantly different. Fv/Fm means efficiency of PSII, FPSII means quantum yield of PSII, qP means photochemical quenching, and NPQ means non-photochemical quenching.

Table 5
Impact of foliar application with micronutrients (MNs) or germinated maize grains extract (gMGE) on gas exchange traits of a wild tomato species (line 0043–1) and two tomato cultivars [Castle Rock (CR) and C10] that differed in their tolerance to salinity and growing under adverse conditions of 9 dS m^{-1} NaCl.

Source of variation	Net photosynthesis rate (*Pn*; mmol m^{-2} s^{-1})	CO_2 assimilation (*A*; mmol CO_2 m^{-2} s^{-1})	Stomatal conductance (*gs*; mmol CO_2 m^{-2} s^{-1})	Transpiration rate (*E*; mmol H_2O m^{-2} s^{-1})
Salinity (S)	*	*	**	*
- NaCl	12.5^{a} ± 0.2	15.0^{a} ± 0.3	356^{a} ± 7	1.96^{a} ± 0.05
+ NaCl (9 dS m^{-1})	8.8^{b} ± 0.2	10.5^{b} ± 0.2	229^{b} ± 5	1.28^{b} ± 0.03
Cultivars/species (CS)	*	*	**	*
CR	8.8^{c} ± 0.2	10.1^{c} ± 0.2	229^{c} ± 4	1.25^{c} ± 0.03
C10	10.6^{b} ± 0.2	12.6^{b} ± 0.3	276^{b} ± 5	1.50^{b} ± 0.04
0043–1	12.5^{a} ± 0.3	15.6^{a} ± 0.4	372^{a} ± 8	2.12^{a} ± 0.06
Promoters (Pr)	*	*	*	*
Control (without)	9.8^{c} ± 0.2	12.1^{c} ± 0.2	276^{c} ± 5	1.53^{b} ± 0.04
MNs	10.6^{b} ± 0.2	12.7^{b} ± 0.2	290^{b} ± 6	1.59^{b} ± 0.04
gMGE	11.5^{a} ± 0.3	13.6^{a} ± 0.3	311^{a} ± 6	1.75^{a} ± 0.05
S × CS × Pr	*	*	*	*

* indicates differences at $P \leq 0.05$ and ** indicates differences at $P \leq 0.01$ probability level. In each column, means with different letters are considered significantly different.

Table 6
Impact of foliar application with micronutrients (MNs) or germinated maize grains extract (gMGE) on relative content of water (RWC) and osmoprotectants contents of a wild tomato species (line 0043–1) and two tomato cultivars [Castle Rock (CR) and C10] that differed in their tolerance to salinity and growing under adverse conditions of 9 dS m^{-1} NaCl.

Source of variation	RWC (%)	Proline(μg g^{-1} FW)	Soluble sugars (mg g^{-1} FW)	Glycine betaine (μg g^{-1} FW)
Salinity (S)	*	**	*	*
- NaCl	80.6^{a} ± 6.2	31.7^{b} ± 0.6	7.18^{b} ± 0.16	5.15^{b} ± 0.12
+ NaCl (9 dS m^{-1})	60.8^{b} ± 4.7	44.2^{a} ± 0.8	9.34^{a} ± 0.21	5.86^{a} ± 0.15
Cultivars/species (CS)	*	*	*	*
CR	65.2^{c} ± 4.8	33.9^{c} ± 0.6	7.06^{c} ± 0.15	4.99^{c} ± 0.10
C10	70.1^{b} ± 5.7	37.3^{b} ± 0.7	8.37^{b} ± 0.20	5.51^{b} ± 0.13
0043–1	76.8^{a} ± 5.9	42.6^{a} ± 0.8	9.36^{a} ± 0.21	6.02^{a} ± 0.17
Promoters (Pr)	*	*	*	*
Control (without)	68.7^{b} ± 5.1	35.9^{b} ± 0.6	7.86^{b} ± 0.17	5.33^{b} ± 0.13
MNs	70.3^{ab} ± 5.4	37.3^{b} ± 0.7	8.20^{b} ± 0.18	5.50^{ab} ± 0.13
gMGE	73.1^{a} ± 5.8	40.6^{a} ± 0.8	8.73^{a} ± 0.20	5.69^{a} ± 0.15
S × CS × Pr	*	*	*	*

* indicates differences at $P \leq 0.05$ and ** indicates differences at $P \leq 0.01$ probability level. In each column, means with different letters are considered significantly different.

foliar application with gMGE significantly increased all of the parameters mentioned above compared to foliar application with MNs, which in turn significantly increased all of these parameters compared to the control. Foliar-applied gMGE increased *Pn*, *A*, *gs*, *E*, RWC, proline content, soluble sugars content, and GB content by 17.3, 12.4, 12.7, 14.4, 6.4, 13.1, 11.1, and 6.8%, respectively comparing with the control. For the interaction of the three factors; salt stress, tomato genotypes, and promoters' application, significant differences were noticed among the combined treatments for all of the above-mentioned indices (Tables 5 and 6).

3.3. Effects on markers of oxidative stress (superoxide; $O_2^{\cdot-}$ and hydrogen peroxide; H_2O_2), tissue cell integrity, and some of antioxidant system components

For saline stress, saline irrigation of tomato plants noticeably elevated $O_2^{\cdot-}$, H_2O_2, lipid peroxidation (assessed as malondialdehyde; MDA), EL, ascorbate (AsA), and glutathione (GSH) levels, and catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) activities by 62.9, 67.3, 61.0, 162.3, 70.6, 79.2, 36.1, 38.2, and 41.7%, respectively, while stability index of mem-

branes (MSI) was significantly decreased by 51.5%, respectively compared to non-salty irrigation (Tables 7 and 8). Regarding the genotypes of tomatoes, 0043–1 genotype had significant decreases in the levels of $O_2^{\cdot-}$, H_2O_2, MDA, and EL, while had increases in MSI, AsA and GSH levels, and CAT, APX, and GR activities compared to C10 genotype, which in turn outperformed CR genotype with respect to these parameters. The wild genotype (0043–1) had decreases in the levels of $O_2^{\cdot-}$, H_2O_2, MDA, and EL by 30.4, 34.3, 30.1, and 59.1%, respectively, while had increases in MSI, AsA and GSH levels, and CAT, APX, and GR activities by 39.2, 63.2, 37.1, 20.8, 29.9, and 33.0% compared to CR genotype. Regarding the promoters' application, outperforming MNs significantly, foliar application with gMGE significantly decreased the levels of $O_2^{\cdot-}$, H_2O_2, MDA, and EL by 14.8, 21.1, 27.0, and 32.2%, respectively, while increased MSI, AsA and GSH levels, and CAT, APX, and GR activities by 10.8, 19.1, 19.7, 9.9, 12.2, and 18.1% comparing with the control. For the interaction of the three factors; salt stress, tomato genotypes, and promoters' application, significant differences were detected among the combined treatments for all of the above-mentioned parameters (Tables 7 and 8).

3.4. Effects on activities of superoxide dismutases (SODs) and related MNs contents

Regarding saline stress, saline watering of tomato plants significantly increased the activities of superoxide dismutases (Fe-SOD, Mn-SOD, Zn/Cu-SOD, and total SOD) by 41.2, 16.9, 40.9, and 27.2%, respectively, while related MNs (Fe, Mn, and Zn + Cu) contents were decreased by 16.2, 18.2, and 23.2%, respectively compared to non-salty irrigation (Table 9). Regarding tomato genotypes, 0043–1 genotype had significant increases in Fe-SOD, Mn-SOD, Zn/Cu-SOD, and total SOD activities and related Fe, Mn, and Zn + Cu contents compared to C10 genotype, which in turn significantly outperformed CR genotype with respect to these parameters. The wild genotype (0043–1) had increases in Fe-SOD, Mn-SOD, Zn/Cu-SOD, and total SOD activities and related Fe, Mn, and Zn + Cu contents by 27.8, 15.4, 34.8, 21.9, 18.3, 22.9, and 29.5% compared to CR genotype. Regarding the promoters' application, outperforming MNs, foliar application with gMGE significantly increased the parameters mentioned above by 27.8, 9.0, 20.8, 14.7, 9.5, 12.2 and 12.8% comparing with the control. For the interaction of the three factors; salt stress, tomato genotypes, and promoters' application, significant differences were observed among the combined treatments for all of the above-mentioned parameters (Table 9).

3.5. Effects on contents and ratio of potassium (K^+) and sodium (Na^+)

For saline stress, saline irrigation of tomato plants significantly increased Na^+ content by 631.8%, while content of K^+ and ration of K^+/Na^+ were noticeably decreased by 19.3 and 88.0%, respectively compared to non-salty irrigation (Table 10). Regarding the genotypes of tomatoes, 0043–1 genotype had increases in K^+ and Na^+ contents by 18.9 and 45.9%, while had decrease in K^+/Na^+ ratio by 23.7%, on the other hand, C10 genotype had reduction in Na^+ content by 24.9%, while had increase in K^+/Na^+ ratio by 17.8% compared to CR genotype. Regarding the promoters' application, outperforming MNs significantly, foliar application with gMGE significantly decreased Na^+ content by 34.3%, while significantly elevated content of K^+ and ratio of K^+/Na^+ by 16.0, and 34.6%, respectively, comparing with the control. For the interaction of the three factors; salt stress, tomato genotypes, and promoters' application, significant differences were detected among the combined treatments for the above-mentioned parameters (Table 10).

4. Discussion

As severe environmental abiotic stress that extends all over the world, salinity prevents plants from achieving their full physiological, biochemical, and genetic potential, resulting in disturbances to their growth, development, and productivity based on the severity and duration of exposure to stress conditions of salinity (Torche et al., 2018; Desoky et al., 2019). As a response to salt stress, in our study, the genotypes of tomatoes demonstrated less or more relative tolerance based on local varieties or wild lines. This is due to a complex mixture of phenological, physiological and biochemical responses, which were expressed by a relative reduction in growth and growth-related indices and relative increased production of low-molecular-mass and enymatic antioxidant defense system, causing economic losses (Torche et al., 2018).

Genetic variations found in wild genotypes have been used to boost salt tolerance in different cultivars (Zaki and Yokoi, 2016). The wild line "0043–1" has a high salt tolerance (Zaki and Yokoi, 2016), while the CR cultivar is more sensitive to salt stress than C10 cultivar (Al-Daej, 2018), so they were chosen to implement the current study.

As is known, MNs boost tolerance to salinity stress in plants by reducing oxidative stress through some mechanisms, including minimizing the toxicity of ions and keeping water balance, improving the uptake and assimilation of essential mineral nutrients, modifying the attributes of gas exchange, bio-synthesizing the compatible solutes and plant hormones, and modifying genetic

Table 7
Impact of foliar application with micronutrients (MNs) or germinated maize grains extract (gMGE) on oxidative stress markers and their consequences for lipid peroxidation (MDA), leakage of ions/electrolytes (EL) and stability index of cell membranes (MSI) of a wild tomato species (line 0043–1) and two tomato cultivars [Castle Rock (CR) and C10] that differed in their tolerance to salinity and growing under adverse conditions of 9 dS m^{-1} NaCl.

Source of variation	$O_2^{\cdot-}$ (μmol g^{-1} FW)	H_2O_2 (μmol g^{-1} FW)	MDA (μmol g^{-1} FW)	EL (%)	MSI (%)
Salinity (S)	*	**	*	**	**
- NaCl	1.05^b ± 0.02	2.94^b ± 0.04	10.0^b ± 0.1	5.3^b ± 0.3	72.8^a ± 4.9
+ NaCl (9 dS m^{-1})	1.71^a ± 0.03	4.92^a ± 0.08	16.1^a ± 0.2	13.9^a ± 0.8	35.3^b ± 4.1
Cultivars/species (CS)	*	*	*	*	*
CR	1.58^a ± 0.03	4.69^a ± 0.07	15.3^a ± 0.2	12.7^a ± 0.7	57.4^c ± 3.6
C10	1.46^b ± 0.03	4.02^b ± 0.06	13.3^b ± 0.2	11.0^b ± 0.6	63.7^b ± 4.0
0043–1	1.10^c ± 0.02	3.08^c ± 0.05	10.7^c ± 0.1	5.2^c ± 0.3	79.9^a ± 5.9
Promoters (Pr)	*	*	*	*	*
Control (without)	1.49^a ± 0.02	4.40^a ± 0.07	15.2^a ± 0.2	11.5^a ± 0.6	63.7^c ± 4.2
MNs	1.39^b ± 0.02	3.92^b ± 0.06	12.9^b ± 0.2	9.7^b ± 0.6	66.7^b ± 4.5
gMGE	1.27^c ± 0.02	3.47^c ± 0.05	11.1^c ± 0.1	7.8^c ± 0.4	70.6^a ± 4.8
S × CS × Pr	*	*	*	*	*

* indicates differences at $P \leq 0.05$ and ** indicates differences at $P \leq 0.01$ probability level. In each column, means with different letters are considered significantly different.

Table 8
Impact of foliar application with micronutrients (MNs) or germinated maize grains extract (gMGE) on some of antioxidant defense system components of a wild tomato species (line 0043–1) and two tomato cultivars [Castle Rock (CR) and C10] that differed in their tolerance to salinity and growing under adverse conditions of 9 dS m^{-1} NaCl.

Source of variation	Ascorbic acid (nmol g^{-1} FW)	GSH (nmol g^{-1} FW)	CAT activity (Unit mg^{-1} protein)	APX activity (Unit mg^{-1} protein)	GR activity (Unit mg^{-1} protein)
Salinity (S)	**	**	*	*	*
- NaCl	$5.61^{b} \pm 0.10$	$2.36^{b} \pm 0.04$	$29.6^{b} \pm 0.5$	$12.3^{b} \pm 0.2$	$8.4^{b} \pm 0.2$
+ NaCl (9 dS m^{-1})	$9.57^{a} \pm 0.17$	$4.23^{a} \pm 0.07$	$40.3^{a} \pm 0.6$	$17.0^{a} \pm 0.3$	$11.9^{a} \pm 0.3$
Cultivars/species (CS)	*	*	*	*	*
CR	$5.95^{c} \pm 0.10$	$2.78^{c} \pm 0.05$	$31.8^{c} \pm 0.5$	$12.7^{c} \pm 0.3$	$8.8^{c} \pm 0.2$
C10	$7.12^{b} \pm 0.13$	$3.30^{b} \pm 0.06$	$34.7^{b} \pm 0.5$	$14.7^{b} \pm 0.3$	$10.0^{b} \pm 0.3$
0043–1	$9.71^{a} \pm 0.17$	$3.81^{a} \pm 0.06$	$38.4^{a} \pm 0.6$	$16.5^{a} \pm 0.3$	$11.7^{a} \pm 0.2$
Promoters (Pr)	*	*	*	*	*
Control (without)	$6.97^{c} \pm 0.12$	$2.99^{c} \pm 0.05$	$33.4^{b} \pm 0.5$	$13.9^{b} \pm 0.3$	$9.4^{c} \pm 0.2$
MNs	$7.51^{b} \pm 0.13$	$3.32^{b} \pm 0.06$	$34.8^{b} \pm 0.5$	$14.5^{b} \pm 0.3$	$10.0^{b} \pm 0.3$
gMGE	$8.30^{a} \pm 0.15$	$3.58^{a} \pm 0.06$	$36.7^{a} \pm 0.6$	$15.6^{a} \pm 0.3$	$11.1^{a} \pm 0.3$
S × CS × Pr	*	*	*	*	*

* indicates differences at $P \leq 0.05$ and ** indicates differences at $P \leq 0.01$ probability level. In each column, means with different letters are considered significantly different.

Table 9
Impact of foliar application with micronutrients (MNs) or germinated maize grains extract (gMGE) on activity of superoxide dismutases (SODs) and related MNs contents of a wild tomato species (line 0043–1) and two tomato cultivars [Castle Rock (CR) and C10] that differed in their tolerance to salinity and growing under adverse conditions of 9 dS m^{-1} NaCl.

Source of variation	Fe content mg g^{-1} DW	Mn content	Zn + Cu content	Fe-SOD activity Unit mg^{-1} protein	Mn-SOD activity	Zn/Cu-SOD activity	Total SOD activity
Salinity (S)	*	*	*	*	*	*	*
- NaCl	$523^{a} \pm 10$	$428^{a} \pm 7$	$323^{a} \pm 6$	$1.7^{b} \pm 0.1$	$6.5^{b} \pm 0.2$	$2.2^{b} \pm 0.01$	$10.3^{b} \pm 0.4$
+ NaCl (9 dS m^{-1})	$438^{b} \pm 8$	$350^{b} \pm 6$	$248^{b} \pm 4$	$2.4^{a} \pm 0.2$	$7.6^{a} \pm 0.3$	$3.1^{a} \pm 0.2$	$13.1^{a} \pm 0.5$
Cvs./sp. (CS)	*	*	*	*	*	*	*
CR	$442^{c} \pm 8$	$353^{c} \pm 6$	$254^{c} \pm 4$	$1.8^{c} \pm 0.1$	$6.5^{c} \pm 0.2$	$2.3^{c} \pm 0.2$	$10.5^{c} \pm 0.4$
C10	$478^{b} \pm 9$	$381^{b} \pm 6$	$275^{b} \pm 5$	$2.0^{b} \pm 0.2$	$7.1^{b} \pm 0.3$	$2.7^{b} \pm 0.2$	$11.8^{b} \pm 0.4$
0043–1	$523^{a} \pm 11$	$434^{a} \pm 7$	$329^{a} \pm 6$	$2.3^{a} \pm 0.2$	$7.5^{a} \pm 0.3$	$3.1^{a} \pm 0.2$	$12.8^{a} \pm 0.6$
Promoters (Pr)	*	*	*	*	*	*	*
Control (without)	$454^{b} \pm 9$	$362^{b} \pm 6$	$265^{b} \pm 5$	$1.8^{b} \pm 0.1$	$6.7^{b} \pm 0.2$	$2.4^{c} \pm 0.1$	$10.9^{c} \pm 0.4$
MNs	$491^{a} \pm 9$	$400^{a} \pm 7$	$294^{a} \pm 5$	$2.0^{b} \pm 0.1$	$7.0^{ab} \pm 0.3$	$2.7^{b} \pm 0.2$	$11.8^{b} \pm 0.4$
gMGE	$497^{a} \pm 10$	$406^{a} \pm 7$	$299^{a} \pm 6$	$2.3^{a} \pm 0.2$	$7.3^{a} \pm 0.3$	$2.9^{a} \pm 0.2$	$12.5^{a} \pm 0.5$
S × CS × Pr	*	*	*	*	*	*	*

* indicates differences at $P \leq 0.05$ probability level. In each column, means with different letters are considered significantly different.

Table 10
Impact of foliar application with micronutrients (MNs) or germinated maize grains extract (gMGE) on contents and ratio of K^+ and Na^+ of a wild tomato species (line 0043–1) and two tomato cultivars [Castle Rock (CR) and C10] that differed in their tolerance to salinity and growing under adverse conditions of 9 dS m^{-1} NaCl.

Source of variation	K^+ content mg g^{-1} DW	Na^+ content	Ratio of K^+/Na^+
Salinity (S)	*	**	**
- NaCl	$22.3^{a} \pm 1.6$	$0.88^{b} \pm 0.07$	$26.6^{a} \pm 2.3$
+ NaCl (9 dS m^{-1})	$18.0^{b} \pm 1.3$	$6.44^{a} \pm 0.52$	$3.18^{b} \pm 0.3$
Cultivars/species (CS)	*	*	*
CR	$18.5^{c} \pm 1.4$	$3.42^{b} \pm 0.26$	$15.2^{b} \pm 1.3$
C10	$20.0^{b} \pm 1.3$	$2.57^{c} \pm 0.21$	$17.9^{a} \pm 1.6$
0043–1	$22.0^{a} \pm 1.6$	$4.99^{a} \pm 0.41$	$11.6^{c} \pm 0.9$
Promoters (Pr)	*	*	*
Control (without)	$18.7^{c} \pm 1.3$	$4.34^{a} \pm 0.31$	$13.0^{c} \pm 1.1$
MNs	$20.0^{b} \pm 1.4$	$3.79^{b} \pm 0.33$	$14.1^{b} \pm 1.2$
gMGE	$21.7^{a} \pm 1.5$	$2.85^{c} \pm 0.24$	$17.5^{a} \pm 1.6$
S × CS × Pr	*	*	*

* indicates differences at $P \leq 0.05$ and ** indicates differences at $P \leq 0.01$ probability level. In each column, means with different letters are considered significantly different.

expressions (Hussain et al., 2018; Noreen et al., 2018). Despite these advantages of MNs, gMGE surpassed MNs in enhancing the tolerance of salinity in the three tomato genotypes, especially the wild line "0043–1". The gMGE containing biostimulants (Table 1) contributed to some potential mechanisms for stressful tomato plants to boost their tolerance to be able to defend themselves against saline stress. One of them, osmoprotectant compounds (e.g., soluble sugars, free proline, and glycine betaine; GB), which are known to prevent water loss from plant tissues to maintain cellular turgidity, membrane stability, and efficient metabolic processes against oxidative stress (Rady et al., 2016; Rady et al., 2018). Antioxidants (e.g., AsA and GSH) and antioxidant powerful activity (84.6%; Table 1) detected in gMGE represent another potential mechanism against oxidative stress effects in plants (Aliniaeifard et al., 2016; Rady and Hemida, 2016). A third possible mechanism is plant hormones detected in gMGE that possibly maintain hormonal balance and enhance the antioxidant defense system in stressful plants against oxidative stress (Kaya et al., 2009). All of these mechanisms along with MNs (e.g., Fe, Mn, Zn, and Cu) that are detected at massive concentrations in gMGE

(Table 1) in association with other mechanisms not mentioned here make the gMGE an effective potent strategy for protecting stressful plants against salt stress-stimulated oxidative stress (Semida and Rady, 2014; Rady et al., 2019b).

The cellular pathways and plant metabolism are seriously damaged by oxidative stress-stimulated osmotic and ionic stresses, which are catalyzed by saline stress, thereby inhibiting the growth and development of plants (Liang et al., 2018; Frukh et al., 2020). Among the stages of the plant's life cycle, early seedling growth is one of the most sensitive stages to salt stress (Munns and Tester, 2008). In this investigation, saline stress decreased the growth of the three tomatoe genotypes, where the local cultivar; CR was the most affected genotype, while the wild line "0043–1" was the least affected genotype.

This detrimental effect of saline stress on tomato growth may be ascribed to the improvement in overproduction of different species of reactive oxygen (ROS), especially H_2O_2 and $O_2^{\cdot-}$ (Table 7). However, tomato plants foliar-treated with MNs or gMGE showed a higher growth level (fresh and dry weights of plant shoots and roots), with gMGE outperforming MNs, than untreated ones (Table 3). This positive result can be attributed to the active ingredients with low molecular mass and high molecular mass (enzymes) of the plant's antioxidant defense system that are enhanced by the application of these promoters, especially gMGE (Tables 6, 8 and 9), which have considerably reduced the biomarkers of oxidative stress ($O_2^{\cdot-}$ and H_2O_2; Table 7) side by side with enhanced photosynthetic capacity, including hill reaction activity (Table 4) and gas exchange traits (Table 5). Beyond the MNs, promoting the growth of salty stressful tomatoes using gMGE can be ascribed to the biologically active ingredients present in the gMGE (Table 1). These active ingredients likely represent some of the mechanisms that stimulates plant growth and may participate in mitigating the detrimental effects of salinity. Among these growth-promoting substances that can penetrate into the plant as a result of the application of gMGE, cytokinins (CKs) play pivotal roles in promoting division and elongation of plant cells, activating chloroplast protein synthesis, enhancing gene expression of enymes related to chlorophyll biosynthesis, accelerating chloroplast differentiation, stimulating carbohydrate metabolism, and creating new source-sink relationships, all of which lead to increased accumulation of dry matter in tomato plants. In addition, auxins and gibberellins (GAs) promote plant cell performance (development and growth) and lateral roots (Taiz and Zeiger, 2010).

In the present study, salinity-induced inhibition in plant growth was associated with decreased photosynthetic capacity/efficiency [e.g., total chlorophylls and carotenoids, chlorophyll fluorescence, activity of hill reaction (Table 4), and gas exchange traits such as rates of net photosynthesis, CO_2 assimilation and transpiration, and stomatal conductance (Table 5)]. Sui et al. (2010) obtained consistent results. Mechanisms that are donated to plants by applying gMGE, especially plant hormones and MNs, increase stay greenness by keeping chlorophyll contents and delaying senescence of leaves by enhancing the capacity/efficiency of photosynthesis to donate vigorous growth to a stressful plant. The content of chlorophylls is one of the most master physiological indicators performing the capacity/efficiency of photosynthesis in plants (Desoky et al., 2019; Rady et al., 2019d) and an increase in chlorophyll level under saline stress can be a biochemical index of salinity tolerance (Stefanov et al., 2016). In the present study, gMGE application significantly elevated the capacity/efficiency of photosynthesis in salt-stressed plants (Tables 4 and 5), including levels of chlorophylls that most likely attributed to the favorable impact of gMGE on ionic homeostasis (Tables 9 and 10). It suppressed the uptake and content of Na^+ (Table 10), suppressing its harmful impact on chlorophyll biosynthesis (Rady et al., 2019b). Additionally, It is likely that the simultaneous increase in the content of carotenoids in promoters (especially gMGE)-treated tomato plants subjected to the stress conditions of salinity (Table 4) is closely connected to improved the tolerance to salinity stress in plants. As reported in Rady et al., 2019b, carotenoids are antioxidants that are able to protect photosynthetic machinery from damage of photo-inhibitory induced by $^1O_2$ (single oxygen as one of ROS) and suppress the excited state of chlorophyll.

There is some evidence to support applying gMGE to promote salt tolerance in saline-affected plants by stimulating a number of compatible osmolytes such as free amino acids (including proline), soluble sugars, and potassium (K^+) (Semida and Rady, 2014; Rady et al., 2019b). These results confirm the results (e.g., free proline, soluble sugars, glycine betaine; GB, and K^+) of the current study, where salt stress increased the contents of osmoprotectants (except K^+, which was declined), however, the application of gMGE or MNs (with outperforming of gMGE) further increased osmoprotectant contents in saline-affected tomato plants (Tables 6 and 10). Under the saline conditions of this study, tomato plants (with the superiority of the wild line "0043-1″) attempted to maintain the osmotic homeostasis of their cells by increasing their contents of osmoprotectant compounds to reduce osmotic stress and increase their relative water content (RWC). However, the application of tested promoters, especially gMGE, increased the contents of these osmoprotectants by increasing their biosynthesis and accumulation for plant survival in such harmful conditions. This result indicates that the contents of osmoprotectants in growing tissues is a pivotal indicator for assessing the tolerance to salt injuries in plants (Semida and Rady, 2014; Rady et al., 2019b). As an effective mechanism, these promoters (especially gMGE)-increased osmoprotectant compounds (as integral part of salt tolerance) retained high RWC and maintained high cellular membranes stabilities (MSI) under salt stress, and thus healthy metabolic processes (Semida and Rady, 2014). Outperforming MNs, gMGE elevated the accumulation of osmoregulation compounds (Tables 6 and 10) to adjust cellular osmotic pressure to maintain cellular turgor pressure and protect cell membranes from damage of harmful salts (Rady et al., 2019b). In this regard, Kavi Kishor et al. (2005) and Szabados and Savouré (2009) cconcluded that proline probably increases water influx or reduces its efflux to provide cellular turgor necessary for cell expansion, as well as proline is able to scavenge ROS, and thus has a role in stabilizing cell membranes and protecting cell functions. Besides, as it is known that soluble sugars are a potent osmotic substance, GB is also an effective osmoprotectant compound as it improves osmotic pressure and water use efficiency in plant tissues, increases water availability for stressful plant growth and balanced nutrient uptake including K^+, and improves nutrients' solubility in plant cells to maximizing metabolic mechanisms and synthesizing more antioxidants in stressful plants (Rady et al., 2018). These increases in osmoprotectant compounds in promoters (especially gMGE)-treated tomato plants act as synergists with the active ingredients with low-molecular-mass and high-molecular-mass (enzymes) of the stressed plant's antioxidant defense system (Tables 8 and 9), suggesting high potential for ROS scavenging to provide a structural protection of thylakoid membranes from ROS' attack, resulting in more potency for gMGE-applied plants against excessive salt injuries on chlorophylls content.

Under saline stress conditions, plants are forced to overproduce ROS (especially $O_2^{\cdot-}$ and H_2O_2 that are main species) within their cells, resulting in increased lipid peroxidation (assessed as malondialdehyde; MDA) and electrolyte leakage (EL) and decreased MSI (Table 7). These results are found to cause necrosis or may apoptosis of plant leaves (Liu et al., 2020). In our study, the increased levels of $O_2^{\cdot-}$ and H_2O_2 occurred due to salt stress-stimulated oxidative stress caused significant decline in CO_2 fixation (Table 5) and ultimately, biomass accumulation in terms of decreased plant

growth (Table 3). It may lead to higher leakage of electrons to O_2 to form $O_2^{\cdot-}$ that is dismutated to form H_2O_2. Mostly, MDA is used as a marker to indicate lipid peroxidation levels in cellular membranes and plant response to stress conditions (Juknys et al., 2012). This indicates that the wild line "0043–1" is more salt-tolerant than other local tomato cultivars because it has a lower level of MDA under the stress conditions of salinity. Otherwise, application of the tested promoters (especially gMGE) stimulated the three tested tomato genotypes to be more salt-tolerant (with the superiority of the wild line "0043–1") by reducing $O_2^{\cdot-}$ and H_2O_2 levels, and thus reducing the levels of MDA and EL and increasing MSI (Table 7). To control ROS as occurred in the prsesnt study by applied promoters (especially gMGE), various intrinsic mechanisms in tomato plants were strengthened to maintain healthy metabolic pathways under salt stress. Among these mechanisms, the effective low-molecular-mass and high-molecular-mass (enzymes) components of the plant's antioxidant defense system (Tables 8 and 9), which play most roles in scavenging the overproduced ROS during saline stress conditions to maintain cellular homeostasis in the plant. Semida and Rady (2014), You and Chan (2015), and Rady et al. (2019b) obtained consistent results. Among these antioxidative components, ascorbate (AsA) and glutathione (GSH) as non-enzymatic antioxidants and catalase (CAT), AsA peroxidase (APX), GSH reductase (GR) and superoxide dismutases (SODs) as enzymatic antioxidants, whose activities have increased under salt stress, but induced antioxidant activities were higher in the wild line "0043–1" than in other local tomato cultivars that might be due to better ROS (especially $O_2^{\cdot-}$ and H_2O_2) scavenging ability in this wild line. However, outweighing the MNs, gMGE application further increased the activities of these antioxidative (enzymatic and non-enzymatic) components, and suppressed ROS levels by more scavenging of $O_2^{\cdot-}$ and H_2O_2 in the presence of SODs to dismutate $O_2^{\cdot-}$ to H_2O_2, which in turn converted to H_2O and O_2 in the presence of CAT and APX, hence healthy cellular functions. Besides, GR provides tolerance to saline-stressed plants by scavenging ROS and their reaction products through the AsA-GSH pathways. AsA is one of the most effective ROS scavengers because it is able to contribute its electrons in favor of many reactions (e.g., enzymatic and non-enzymatic), and to protect cellular membranes by eliminating $O_2^{\cdot-}$ and OH^- radicals, directly, and by regeneration of α-tocopherol from the tocopheroxyl radical, and the oxidized form of AsA; dehydroascorbate can be reduced chemically by GSH to AsA (Foyer and Halliwell, 1976). To withstand salt stress effects, tomato plants had a fruitful synergy among these antioxidantive (enzymatic; CAT, APX, GR, and SODs, and non-enzymatic; AsA and GSH) components to scavenge ROS effectively. In the present study, among the three tomato genotypes, plants of the wild line "0043–1" were the most salt-tolerant because they showed the most improved activities of antioxidative defense system components (Tables 8 and 9).

Under conditions of saline stress, the activities of all SOD forms (e.g., Fe-SOD, Mn-SOD, and Zn/Cu-SOD) and total SODs were increased at the same time as the micronutrient (e.g., Fe, Mn, Zn, and Cu) contents were reduced (Table 9). This result may be attributed to the incorporation of these micronutrients into SODs for their activities to increase the $O_2^{\cdot-}$ scavenging function. Compared to normal conditions, Fe-SOD and Zn/Cu-SOD awarded higher activities (41.2 and 40.9%, respectively) under salt stress than Mn-SOD and total SODs activities (16.9 and 27.2%, respectively) (Table 9). This result may be due to the use of Fe-SOD and Zn/Cu-SOD in $O_2^{\cdot-}$ scavenging more than Mn-SOD. Compared to the local tomato cultivars (CR and C10), the wild line "0043–1" showed relatively lower percentage of growth reduction (Table 3) corresponded to higher activities of Fe-SOD, Mn-SOD, and Zn/Cu-SOD in its leaf tissues (Table 9) and lower levels of oxidative stress biomarkers, including $O_2^{\cdot-}$, which indicate that this wild line is more salt-tolerant. Increased SODs activities under salt stress may be an indication of excessive ROS production and/or a protective mechanism to prevent or at least reduce oxidative damage. When salt-stressed tomato plants were applied with tested promoters (especially gMGE), data showed excessive stimulation of SODs activities while increasing micronutrient contents (especially in the wild line "0043–1") (Table 9) that can give plants additional tolerance to salt-induced oxidative stress. To date, it has been demonstrated that excessive expression of SODs genes gives plants a degree of tolerance to oxidative stress stimulated by various environmental stresses (Yu and Rengel, 1999). However, the genotype used, the degree of environmental stress, the promoter used and its application method are key factors determining the success of agricultural sustainability.

Under conditions of saline stress, the content of K^+ and the ratio of K^+/Na^+ were noticeably decreased with elevated Na^+ accumulation in tomato plants. However, treating plants with tested promoters, especially gMGE reversed the previous trend significantly; increasing K^+ content and K^+/Na^+ ratio while reducing Na^+ accumulation (Table 10), which is connected with tolerance to salinity in plants, especially in the wild line "0043–1", which collected higher content and ratio of K^+ and K^+/Na^+, respectively along with lower Na^+ accumulation in its tissues, and thus more salt tolerance than the local tomato cultivars (CR and C10). Nutrients also play important roles for plants in performing their routine and basic cellular activities during their growth and development. Improvements in nutrient gain in gMGE-treated plants may be due to gMGE's richness in many nutrients. (Table 1). Besides, the good positioning and good regulation of ion absorption can be attributed to the significant improvement in cell MSI associated with the significant suppression levels of EL and MDA (Table 7) of gMGE-treated plants, which in turn stimulate the selectivity of ion uptake and transport.

Finally, beyond the superiority of gMGE application compared to MNs, a higher tolerance to salinity has been approved in the present study for the wild tomato line "0043–1" compared to the local cultivars (CR and C10). This results is consistent with results obtained by Zaki and Yokoi (2016) and Al-Daej (2018). The high tolerance to salinity in the wild line of *Solanum peruvianum* compared to local cultivars of *S. lycopersicum* was clearly demonstrated by the higher levels of antioxidative defense system components, photosynthetic machinery components and K^+/Na^+ ratio, and the lower levels of ROS biomarkers, resulting in higher growth characteristics under the stress conditions of salinity. However, the accumulation of Na^+ was higher in the wild line than in local cultivars under the stress conditions of salinity, indicating the accumulation of ions as one of the salt tolerance mechanisms. This finding can be explained based on the fact that the wild line was more tolerant to salinity, not because it was more able to restrict Na^+ uptake at high salinity levels than local cultivars, but because it had the advantage to withstand high toxic ion levels, including Na^+ in its tissues to be a necessary result of survival (Zaki and Yokoi, 2016). It has been reported in Rush and Epstein (1981) and Zaki and Yokoi (2016) that Na^+ accumulation and Na^+/K^+ ratio in the tissues of wild species can be used as master characteristics in the evaluation of germplasm for salt-tolerance breeding programs of cultivated tomato. In the current study, the wild line "0043–1" as the accession displaying better tolerance to salinity is a good candidate for implication in breeding programs for tolerance to salinity.

5. Conclusions

Based on the findings of this study, the use of gMGE as foliar application has effectively improved the growth of salt-stresses tomato plants through up-regulation of the metabolic pathways

of enzymatic and non-enzymatic antioxidant components, especially forms of SODs, and osmoprotectant compounds. This helped minimize the overproduced ROS and Na^+ accumulation, which synchronized with minimized levels of lipid peroxidation and electrolyte leakage and maximized photosynthetic efficiency, membrane stability index and K^+/Na^+ ratio in gMGE-treated plants, maintaining higher cellular performance and photoprotection. The improved growth of salt-stressed tomato plants due to the improved detoxification of ROS indicates the appropriateness of foliar application to tomato plants using gMGE containing biostimulants. Additionally, since it is more tolerant to salinity compared to local cultivars, the wild tomato line "0043-1" has key characteristics as a good candidate for involvement in breeding programs for tolerance to salinity.

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.

Acknowledgment

This Project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant no. (RG-1-130-40). The authors, therefore, acknowledge with thanks DSR technical and financial support.

References

El-Mageed, Abd, Semida, W.M., Taha, R.S., Rady, M.M., 2018. Effect of summer-fall deficit irrigation on morpho-physiological, anatomical responses, fruit yield and water use efficiency of cucumber under salt affected soil. Scientia Horticulturae 237, 148–155.

Abd El-Mageed, T.A., Rady, M.M., Taha, R.S., Abd El Azeam, S., Simpson, C.R., Semida, W.M., 2020. Effects of integrated use of residual sulfur-enhanced biochar with effective microorganisms on soil properties, plant growth and short-term productivity of *Capsicum annuum* under salt stress. Scientia Horticulturae 261, 108930.

Abou El-Nour, E.A.A., 2002. Growth and nutrient contents response of maize to foliar nutrition with micronutrients under irrigation with saline water. Journal of Biological Sciences 2 (2), 92–97.

Aebi, H., 1984. Catalase in vitro. Methods in Enzymology 105, 121–126.

Al-Daej, M.I., 2018. Salt tolerance of some tomato (*Solanum lycoversicum* L.) cultivars for salinity under controlled conditions. American Journal of Plant Physiology 13, 58–64.

Aliniaeifard, S., Hajilou, J., Tabatabaei, S.J., Sifi-Kalhor, M., 2016. Effects of ascorbic acid and reduced glutathione on the alleviation of salinity stress in olive plants. International Journal of Fruit Science. https://doi.org/10.1080/15538362.2015.1137533.

Alzahrani, Y., Rady, M.M., 2019. Compared to antioxidants and polyamines, the role of maize grain-derived organic biostimulants in improving cadmium tolerance in wheat plants. Ecotoxicology and Environmental Safety 182, 109378.

Anjum, N.A., Sofo, A., Scopa, A., Roychoudhury, A., Gill, S.S., Iqbal, M., Lukatkin, A.S., Pereira, E., Duarte, A.C., Ahmad, I., 2015. Lipids and proteins - major targets of oxidative modifications in abiotic stressed plants. Environmental Science and Pollution Research 22, 4099–4121.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*. Plant Physiol. 24, 1–15.

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39 (1), 205–207.

Bradford, M.N., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 12, 248–254.

Chapman, H.D., Pratt, P.F., 1961. Methods of Analysis for Soil. Plants and Water. University of California, Division of Agricultural Science, Berkeley, CA, USA, pp. 56–63.

Chen, G.X., Asada, K., 1989. Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant and Cell Physiology 30, 987–998.

Desoky, E.M., Merwad, A.M.A., ElSayed, A.I., Rady, M.M., 2019. Stimulating antioxidant defenses, antioxidant gene expression, and salt tolerance in *Pisum sativum* seedling by pretreatment using licorice root extract (LRE) as an organic biostimulant. Plant Physiology and Biochemistry 142, 292–302.

Dionisio-Sese, M.L., Tobita, S., 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135, 1–9.

El-Fouly, M.M., Mobarak, Z.M., Salama, Z.A., 2011. Micronutrients (Fe, Mn, Zn) foliar spray for increasing salinity tolerance in wheat (*Triticum aestivum* L.). African Journal of Plant Science 5 (5), 314–322.

Emilio, A.C., Francisco, P.A., Vicente, M., Manuel, C., Maria, C.B., 1998. Evaluation of salt tolerance in cultivated and wild tomato species through *in vitro* shoot apex culture. Plant Cell, Tissue and Organ Culture 53, 19–26.

Fahad, S., Hussain, S., Bano, A., Saud, S., Hassan, S., Shan, D., Khan, F.A., Khan, F., Chen, Y., Wu, C., Tabassum, M.A., Chun, M.X., Afzal, M., Jan, A., Jan, M.T., Huang, J., 2014a. Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. Environmental Since Pollution Research 22 (7), 4907–4921.

Fahad, S., Hussain, S., Matloob, A., Khan, F.A., Khaliq, A., Saud, S., Hassan, S., Shan, D., Khan, F., Ullah, N., Faiq, M., Khan, M.R., Tareen, A.K., Khan, A., Ullah, A., Ullah, N., Huang, J., 2014b. Phytohormones and plant responses to salinity stress: A review. Plant Growth Regulation 75 (2), 391–404.

Foster, J.G., Hess, J.L., 1980. Responses of Superoxide Dismutase and Glutathione Reductase Activities in Cotton Leaf Tissue Exposed to an Atmosphere Enriched in Oxygen. Plant Physiol. 66, 482–487.

Foyer, C.H., Halliwell, B., 1976. The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta 133, 21–25.

Frary, A., Göl, D., Keles, D., Ökmen, B., Pinar, H., Sigva, H.Ö., Yemenicioglu, A., Doganlar, S., 2010. Salt tolerance in *Solanum pennellii*: antioxidant response and related QTL. BMC Plant Biology 10, 58.

Frukh, A., Siddiqi, T.O., Khan, M.I.R., Ahmad, A., 2020. Modulation in growth, biochemical attributes and proteome profile of rice cultivars under salt stress. Plant Physiology and Biochemistry 146, 55–70.

Giannopolitis, C.N., Ries, S.K., 1977. Superoxide dismutases – occurrence in higher plants. Plant Physiology 59, 309–314.

Giebel, E.P., 2006. Extraction of chloroplasts from plant tissue and their use in demonstrating the Hill Reaction. Chap.3, pp. 31–47. Dept. of Biol. Virginia Commonwealth Univ., 901 West Franklin Street Richmond, Virginia.

Grieve, C.M., Grattan, S.R., 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil 70, 303–307.

Hoagland, D.R., Arnon, D.I., 1950. The Water Culture Method for Growing Plants without Soil. University of California, College of Agriculture, Agricultural Experiment Station, Baltimore, USA.

Huang, C., He, W., Guo, J., Chang, X., Su, P., Zhang, L., 2005. Increased sensitivity to salt stress in an ascorbate-deficient Arabidopsis mutant. J. Exp. Bot. 56, 3041–3049.

Hussain, S., Khalid, M.F., Hussain, M., Ali, M.A., Nawaz, A., Zakir, I., Fatima, Z., Ahmad, S., 2018. Role of Micronutrients in Salt Stress Tolerance to Plants. In: Plant Nutrients and Abiotic Stress Tolerance (Hasanuzzaman, M., Fujita, M., Oku, H., Nahar, K., Hawrylak-Nowak, B., eds.), pp. 363–376, Springer Nature, Singapore Pte Ltd, Singapore.

Irigoyen, J.J., Einerich, D.W., Sánchez-Díaz, M., 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiol. Plantarum 84 (1), 55–60.

Jamil, A., Riaz, S., Ashra, f M., Foolad, M.R., 2011. Gene expression profiling of plants under salt stress. Crit. Rev. Plant Sci. 30, 435–458.

Johnson, C.M., Ulrich, A., 1959. Analytical methods for use in plant analysis. Calif. Agric. Exp. Stn. Bull, No, p. 766.

Juknys, R., Vitkauskaite, G., Racaite, M., Vencloviene, J., 2012. The impacts of heavy metals on oxidative stress and growth of spring barley. Central European Journal of Biology 7, 299–306.

Kabata-Pendias, A., Pendias, H., 1999. Biogeochemistry of Trace Elements. PWN, Warsaw, Poland, p. 398.

Kavi Kishor, P.B., Sangam, S., Amrutha, R.N., SriLaxmi, P., Naidu, K.R., Rao, K.R.S.S., et al., 2005. Review: Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Current Science 88, 424–438.

Kaya, C., Tuna, A.L., Yokaş, I., 2009. The Role of Plant Hormones in Plants Under Salinity Stress. In: Ashraf, M., Ozturk, M., Athar, H. (eds), Salinity and Water Stress, pp. 45-50. Tasks for Vegetation Sciences, vol 44. Springer, Dordrecht.

Kubis, J., 2008. Exogenous spermidine differentially alters activities of some scavenging system enzymes, H_2O_2 and superoxide radical levels in water-stressed cucumber leaves. Journal of Plant Physiology 165, 397–406.

Lavrich, R.J., Hays, M.D., 2007. Validation studies of thermal extraction-GC/MS applied to source emissions aerosols. 1. Semivolatile analyte-nonvolatile matrix interactions. Anal. Chem. 79, 3635–3645.

Lee, S.C., Kim, J.H., Jeong, S.M., Kim, D.R., Ha, J.U., Nam, K.C., 2003. Effect of farinfrared radiation on the antioxidant activity of rice hulls. J. Agric. Food Chem. 51, 4400–4403.

Li, P.-M., Cai, R.-G., Gao, H.-Y., Peng, T., Wang, Z.-L., 2007. Partitioning of excitation energy in two wheat cultivars with different grain protein contents grown under three nitrogen applications in the field. Physiol. Plant. 129, 822–829.

Liang, W., Ma, X., Wan, P., Liu, L., 2018. Plant salt-tolerance mechanism: a review. Biochemical and Biophysical Research Communications 495, 286–291.

Liu, Z., Lei, X., Wang, P., Wang, Y., Lv, J., Li, X., Gao, C., 2020. Overexpression of *ThSAP30BP* from *Tamarix hispida* improves salt tolerance. Plant Physiology and Biochemistry 146, 124–132.

Madhava Rao, K.V., Sresty, T.V.S., 2000. Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. Plant Sci. 157, 113–128.

Martinez-Rodriguez, M.M., Estan, M.T., Moyano, E., Garcia-Abellan, J.O., Flores, F.B., et al., 2008. The effectiveness of grafting to improve salt tolerance in tomato

when an excluder genotype is used as scion. Environmental and Experimental Botany 63, 392–401.
Munns, R., James, R.A., Läuchli, A., 2006. Approaches to increasing the salt tolerance of wheat and other cereals. Journal of Experimental Botany 57 (5), 1025–1043.
Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology 59, 651–681.
Naika, S., Lidt, J., Goffau, M., Hilmi, M., Dam, B., 2005. Cultivation of Tomato Production, Processing and Marketing. Wageningen, Agromisa Foundation and CTA, pp. 1–92.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.
Noreen, S., Fatima, Z., Ahmad, S., Athar, H., Ashraf, M., 2018. Foliar Application of Micronutrients in Mitigating Abiotic Stress in Crop Plants. In: Plant Nutrients and Abiotic Stress Tolerance (Hasanuzzaman, M., Fujita, M., Oku, H., Nahar, K., Hawrylak-Nowak, B., eds.), pp. 95–117, Springer Nature, Singapore Pte Ltd, Singapore.
Osman, A.S., Rady, M.M., 2014. Effect of humic acid as an additive to growing media to enhance the production of eggplant and tomato transplants. The Journal of Horticultural Science and Biotechnology 89, 237–244.
Paradiso, A., Berardino, R., de Pinto, M.C., Sanita di Toppi, L., Storelli, M.M., Tommasi, F., et al., 2008. Increase in ascorbate-glutathione metabolism as local and precocious systemic responses induced by cadmium in durum wheat plants. Plant and Cell Physiology 49, 362–374.
Rady, M.M., 2011a. Effects on growth, yield, and fruit quality in tomato (*Lycopersicon esculentum* Mill.) using a mixture of potassium humate and farmyard manure as an alternative to mineral-N fertilizer. Journal of Horticultural Science & Biotechnology 86 (3), 249–254.
Rady, M.M., 2011b. Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (*Phaseolus vulgaris* L.) plants under salinity and cadmium stress. Scientia Horticulture 129, 232–237.
Rady, M.M., 2012. A novel organo-mineral fertilizer can mitigate salinity stress effects for tomato production on reclaimed saline soil. South African Journal of Botany 81, 8–14.
Rady, M.M., Belal, H.E.E., Gadallah, F.M., Semida, W.M., 2020. Selenium application in two methods promotes drought tolerance in *Solanum lycopersicum* plant by inducing the antioxidant defense system. Scientia Horticulturae 266, 109290.
Rady, M.M., Desoky, E.M., Elrys, A.S., Boghdady, M.S., 2019a. Can licorice root extract be used as effective natural biostimulant for salt-stressed common bean plants? South African Journal of Botany 121, 294–305.
Rady, M.M., Elrys, A.S., Abo El-Maati, M.F., Desoky, E.M., 2019b. Interplaying roles of silicon and proline effectively improve salt and cadmium stress tolerance in *Phaseolus vulgaris* plant. Plant Physiology and Biochemistry 139, 558–568.
Rady, M.M., Hemida, K.A., 2016. Sequenced application of ascorbate-proline-glutathione improves salt tolerance in maize seedlings. Ecotoxicology and Environmental Safety 133, 252–259.
Rady, M.M., Kuşvuran, A., Alharby, H.F., Alzahrani, Y., Kuşvuran, S., 2019c. Pretreatment with proline or an organic bio-stimulant induces salt tolerance in wheat plants by improving antioxidant redox state and enzymatic activities and reducing the oxidative stress. Journal of Plant Growth Regulation 38, 449–462.
Rady, M.M., Rehman, H., 2016. Supplementing organic biostimulants into growing media enhances growth and nutrient uptake of tomato transplants. Scientia Horticulturae 203, 192–198.
Rady, M.M., Taha, R.S., Mahdi, A.H.A., 2016. Proline enhances growth, productivity and anatomy of two varieties of *Lupinus termis* L. grown under salt stress. South African Journal of Botany 102, 221–227.
Rady, M.M., Talaat, N.B., Abdelhamid, M.T., Shawky, B.T., Desoky, E.M., 2019d. Maize (*Zea mays* L.) grains extract mitigates the deleterious effects of salt stress on common bean (*Phaseolusvulgaris* L.) growth and physiology. The Journal of Horticultural Science and Biotechnology 94 (6), 777–789.
Rady, M.O.A., Semida, W.M., Abd El-Mageed, T.A., Hemida, K.A., Rady, M.M., 2018. Up-regulation of antioxidative defense systems by glycine betaine foliar application in onion plants confer tolerance to salinity stress. Scientia Horticulturae 240, 614–622.
Rehman, H., Alharby, H.F., Alzahrani, Y., Rady, M.M., 2018. Magnesium and organic biostimulant integrative application induces physiological and biochemical changes in sunflower plants and its harvested progeny on sandy soil. Plant Physiology and Biochemistry 126, 97–105.
Rush, D.W., Epstein, E., 1981. Comparative studies on the sodium, potassium and chloride relations of a wild halophytic and a domestic salt-sensitive tomato species. Plant Physiology 68, 1308–1313.
Semida, W.M., Rady, M.M., 2014. Presoaking application of propolis and maize grain extracts alleviates salinity stress in common bean (*Phaseolus vulgaris* L.). Scientia Horticulturae 168, 210–217.
Shannon, M.C., Gronwald, J.W., Tall, M., 1987. Effects of salinity on growth and accumulation of organic and inorganic ions in cultivated and wild tomatoes. Journal of the American Society for Horticultural Science 112, 416–423.
Shrivastava, P., Kumar, R., 2015. Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J. Biol. Sci. 22, 123–131.
Singh, H., Kumar, P., Kumar, A., Kyriacou, M.C., Colla, G., Rouphael, Y., 2020. Grafting Tomato as a Tool to Improve Salt Tolerance - a Review. Agronomy 10, 263.
Stefanov, M., Yotsova, E., Rashkov, G., Ivanova, K., Markovska, Y., Apostolova, E.L., 2016. Effects of salinity on the photosynthetic apparatus of two Paulownia lines. Plant Physiology and Biochemistry 101, 54–59.
Sui, N., Li, M., Li, K., Song, J., Wang, B.S., 2010. Increase in unsaturated fatty acids in membrane lipids of *Suaeda salsa* L. enhances protection of photosystem II under high salinity. Photosynthetica 48, 623–629.
Szabados, L., Savouré, A., 2009. Proline: A multifunctional amino acid. Trends in Plant Science 15, 89–97.
Taiz, L., Zeiger, E., 2010. Plant physiology. Sinauer Associates Inc, Sunderland, MA.
Torche, Y., Blair, M., Saida, C., 2018. Biochemical, physiological and phenological genetic analysis in common bean (*Phaseolus vulgaris* L.) under salt stress. Annals of Agricultural Sciences 63, 153–161.
Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Plant Sci. 151, 59–66.
Yang, Y., Guo, Y., 2018. Unraveling salt stress signaling in plants. Journal of Integrative Plant Biology 60, 796–804.
You, J., Chan, Z., 2015. ROS Regulation During Abiotic Stress Responses in Crop Plants - A review. Frontiers in Plant Science 6, 1092.
Yu, Q., Osborne, L.D., Rengel, Z., 1998. Micronutrient deficiency changes activities of superoxide dismutase and ascorbate peroxidase in tobacco plants. Journal of Plant Nutrition 21, 1427–1437.
Yu, Q., Rengel, Z., 1999. Waterlogging influences plant growth and activities of superoxide dismutases in narrow-leafed lupin and transgenic tobacco plants. Journal of Plant Physiology 155, 431–438.
Zaki, H.E.M., Yokoi, S., 2016. A comparative in vitro study of salt tolerance in cultivated tomato and related wild species. Plant Biotechnology (Tokyo) 33, 361–372.
Zhang, P., Senge, M., Dai, Y., 2016. Effects of salinity stress on growth, yield, fruit quality and water use efficiency of tomato under hydroponics system. Reviews in Agricultural Science 4, 46–55.